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THE VAGINAL SMEAR IN DIAGNOSIS OF CARCINOMA OF THE UTERUS *

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A technic for the study of uterine malignancy by means of smears of vaginal secretions was first described by Papanicolaou in 1928.[1] The value of the technic has been demonstrated by Papanicolaou and Traut [2] in a beautifully illustrated monograph setting forth their experience on a large number of patients. Meigs, Graham, Fremont-Smith, Kapnick, and Rawson [3] have also emphasized the importance of this method as a routine diagnostic test.

The acceptability of a new method depends on two things: First, it must be shown to be at least as accurate as the methods already in use; and second, it must have some added advantage such as ease of performance. Both have been claimed for the vaginal smear method for diagnosis of uterine cancer, although Papanicolaou and Traut [2] and Meigs *et al.*[3] stress its place as an accessory method.

In this paper we are primarily interested in the practical aspects of the vaginal smear as a procedure for the routine pathologic laboratory. It is, after all, the practising pathologist who will determine the general usefulness of the test. The successful use of the vaginal smear, either for routine diagnosis or in the control of cancer, depends on an understanding of what can be expected from the method, and of the pitfalls in diagnosis.

The method is simple and if necessary may be performed by the patient. The only requirement concerning the patient is that there should be no interference with the vaginal tract, as from douching or examination, within 12 hours before the sample is taken. The secretions are aspirated from the fornix by a clean, *dry* glass pipette, about 8 inches

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long, provided with a bulb for suction, and are then expelled onto one or more clean, *dry* slides and thinly smeared in a manner similar to the making of blood smears. The slide is immediately placed in a bottle of 95 per cent alcohol, or equal parts of ether and 95 per cent alcohol. After 15 minutes' fixation, the smear is ready to be stained, but an indefinite period in the solution is not harmful. The smear may be dried after fixation and stained at any convenient time, but it is important to avoid drying before fixing. Papanicolaou's stain produces good nuclear detail and is satisfactory. It is important that all of these steps be carefully followed.

The reliability of the method has been reported as extraordinarily high. The figures given by Papanicolaou and Traut [2] and by Meigs and his co-workers [3] correspond closely. The following table shows the small percentage of discrepancy between the diagnoses made of cases by the smear method and by biopsy as reported by these writers.

	Papanicolaou and Traut	*Meigs et al.*
Carcinoma of Fundus		
Number of cases	53	12
Per cent positive by biopsy and negative by smear	9.3*	8.3
Carcinoma of Cervix		
Number of cases	127	46
Per cent positive by biopsy and negative by smear	1.6*	2.2

Meigs and co-workers [3] further reported that in a group of 63 cases, independent diagnoses made by two of the authors accorded perfectly, all the smears being clearly positive or negative, none "suspicious," and that "Of 153 negative cases positive smears were reported in 4—error 2.6 per cent." Papanicolaou and Traut [2] mentioned no false positive diagnoses in their series. Ayre [4] recently reported that 95 per cent of 40 patients with carcinoma of the uterus showed tumor cells in the vaginal smear. These are remarkable records indeed. Certainly material obtained for biopsy, unless the site is very carefully selected, does not give a true picture of the condition of the genital tract in a higher percentage of cases. In spite of this, however, those having the widest experience with the method are cautious in recommending it as a substitute for biopsy or curettage, and have emphasized the need of confirming the diagnosis made on the smear by examination of tissue before therapy is undertaken. This present restriction limits the advantages of the technic as a conclusive diagnostic procedure.

An unexpected and important feature of this method is the demon-

* Excluding 2 post-irradiation cases with negative smears in each group.

stration of carcinoma in its earliest stages before invasion of the stroma has occurred. Some of the patients with carcinoma *in situ* reported by Papanicolaou and Traut [2] had no symptoms and in some cases the biopsies were negative, but the diagnosis of carcinoma was confirmed after hysterectomy. Papanicolaou and Traut stated that their experience is not sufficient to speak uncompromisingly on the accuracy of the diagnosis of early carcinoma, but they nevertheless feel that the "vaginal smear is the only practical method thus far developed which is useful in revealing the very early carcinomatous processes, particularly those of the cervix." If subclinical carcinoma is demonstrable by smears, the method may be of incalculable aid in improving the curability of carcinoma of the uterus. This has been stressed by both Papanicolaou and Traut, and by Meigs.[3] But there are many considerations to be weighed in the diagnosis and treatment of early carcinoma and it may not be out of the way at this point to review them.

Early treatment, the present best hope for the control of cancer, now depends on both the awareness of the patient and the readiness of the physician to investigate symptoms thoroughly. The responsibility of the physician in the delay of treatment has been studied recently by Pack and Gallo [5] and by Harms, Plaut, and Oughterson.[6] In the first of these two reports the patient and physician were implicated in 18.0 per cent of the delays, the physician alone in 17.0 per cent of the delays. The figures in the second report are similar: 27.8 and 17.4 per cent respectively. Unfortunately, it does not seem likely that a physician who, from ignorance or neglect, does not thoroughly investigate a patient with uterine symptoms will make intelligent use of the vaginal smear method. Something certainly can be hoped for from education, both professional and lay, but this approach has its limits.[7]

Periodic examination of well patients is one way to procure early treatment of malignant neoplasms, and cancer control programs have been organized to this end. Only complete, careful statistical studies over a period of years will indicate the profitableness of this approach. Three recent reports of cancer control programs are interesting inasmuch as they vary in organization and special interest. The Strang Cancer Prevention Clinic for Women in New York City organized by L'Esperance [8] has given complete physical examinations and followed patients at intervals of 6 or 12 months. Among 1,103 patients, not all symptomless, examined in 5½ years, there were 7.6 per cent with malignant tumors, the majority of them being early. In a similar clinic at the Memorial Hospital, 1.5 per cent with malignant tumors were found among 263 patients who had no symptoms.[8]

Macfarlane [9] reported the results of periodic pelvic examinations on

well women. Of 1,321 women who were given a single pelvic examination, many returned for further examinations and 255 of them were seen twice yearly for 5 years. Altogether, four carcinomas, all without symptoms, were found as a result of 8,822 examinations over a 5-year period. Three of these carcinomas were found at the first examination, the other one in the fifth year of examinations.

In a study of early carcinoma of the stomach, St. John, Swenson, and Harvey [10] examined fluoroscopically 2,413 patients who had no gastric symptoms. Those who showed abnormalities were followed further, making a total of 2,923 examinations. As a result of this data, partial resection of the stomach was performed on 8 patients. In 5 patients benign lesions were found; but in 3 the lesions were malignant and in 2 consisted of extremely small, early tumors, a carcinoma and a lymphosarcoma; in the third patient there were two malignant neoplasms with a large fungating growth. Metastases were not found in these 3 patients at operation, although the patient with lymphosarcoma died later of generalized disease.

Such studies as these present new problems as to both diagnosis and treatment of early carcinoma, which in view of our limited knowledge require a conservative attitude toward this aspect of malignancy. For example, in the cervix the differentiation of precancerous change, carcinoma *in situ*, and early invasive carcinoma may be a matter of individual judgment in the interpretation of tissue taken for biopsy and impossible from a smear. There is also some evidence that carcinoma *in situ* of the cervix is a sluggish process comparable to Bowen's disease of the skin.[11] Since many of the patients are in the childbearing age and some very young, the diagnosis and treatment of early carcinoma should not be lightly undertaken.

Malignant growth is customarily recognized by a number of alterations of tissue as much as by specific changes in single cells. The pattern and boundaries of a growth are generally considered to be as reliable a guide in determining malignancy as the changes within cells. Frequently hyperplastic or metaplastic cells are difficult to distinguish from neoplastic cells in the absence of tissue relationships. It is therefore necessary to know the benign pathologic variations of cells in question before attempting to diagnose their malignant changes.

The characteristics of the malignant cell have received a great deal of study. Although changes in the cytoplasm of malignant cells have been observed, such as increase in size and density, increase in number, and decrease in size of mitochondria,[12] the visible changes most often described involve the nucleus. The nucleus of the malignant cell tends to be larger than that of the normal cell, especially in relation to

the volume of the cell. The nuclear membrane is often thicker.[12] A rather striking alteration is the increase in the granular material of the nucleoplasm. The large size of one or more nucleoli has been emphasized by most observers of malignant cells, possibly because this is more easily gauged than some of the other aberrations. MacCarty, using fresh tissues removed at operation, first stressed the relationship of nucleolus and nucleus of the malignant as compared to the normal cell.[13-16] Others, using various methods of fixation, have reached the same conclusions as to the relative increase in size of the nucleolus of malignant cells [17-19] and Lewis [12, 20] observed more than the usual amount of nuclear material in malignant cells in tissue culture. Although the visible change in the malignant cell is, to a certain degree, measurable, this should not be taken to indicate that the various modifications are certainly pathognomonic in themselves. Lewis [12] pointed out that malignant cells in culture vary considerably from one to another: "The size, number, position of the nucleoli all vary more or less among cells in the same culture. No two cells are exactly alike." Furthermore, practically all differences noted between normal and abnormal cells are subject to modifications. Our knowledge is yet too fragmentary and inadequate to make a summing up of characteristics more than provisional.[12]

Nevertheless, the observed modifications from the norm have proved to be a practical distinction in diagnosis of malignant cells; for example, the relative size of nucleus and nucleolus as determined by plane or volumetric measurements in normal and pathologic tissue. Foot [17] found the ratio of the diameter of the nucleus to that of the nucleolus to be higher in malignant cells than in normal cells. In malignant cells, the average ratio of nucleolus to nucleus is 0.2 to 0.4, whereas in the normal cell it is below 0.2. MacCarty found that the ratio of nucleolar area to nuclear area varied from 1:5 to 1:17 for malignant cells and 1:13 to 1:45 for nonmalignant cells.[15]

Guttman and Halpern [21] made a great many measurements on benign and malignant tumors and on normal tissues and concluded that the quantitative differences between nuclear-nucleolar volume ratios of normal tissue, hyperplastic tissue, benign and malignant tumors were not significant and that such measurements should be used only as an aid and not as a decisive factor in determining whether or not a tumor cell is malignant. These authors rightly pointed out that nucleoli may be larger in simple hyperplastic tissues than in malignant tissues and that in some malignant tumors the nucleoli may be as small as in normal cells. This serves to emphasize what every pathologist is aware of, namely, that the source and condition of the cells or tissue examined

are important factors and sometimes are essential considerations in evaluating cells. Radiation reaction of tissues may be taken as an example. Both fibroblasts and epithelial cells in irradiated tissue may acquire all the morphologic features of malignant cells and yet remain to all intents and purposes benign. Comparison of cells in malignant and benign growths with the normal cells of their tissue of origin is manifestly more enlightening than comparison of heterogeneous normal tissues and tumors. Lewis [20] observed in tissue cultures that each type of malignant cell usually has one or more characteristics which differentiate it from normal cells. Most pathologists would probably agree with MacCarty's [15] statement: "The cancer cell may not always be distinguished from a normal regenerating cell, but this can be done frequently because there is a difference in volume-relationship between nucleolus, nucleus, and the whole cell in reparative regenerative cells and malignant regenerative cells."

The practicability of differentiation of solitary malignant cells from isolated normal cells in exudates from body cavities was demonstrated long ago, but the accuracy of diagnosis has never been remarkable, probably due in large part to the sample. The figures reported by Foot [17] are instructive. A group of selected cases, checked by biopsy, autopsy, or operation, were examined three times, and the percentage of accuracy given for each of the examinations. In specimens of abdominal fluid in which tumor was present, the percentage of accuracy for the three examinations was 73, 60, and 72, giving an average accuracy of 68 per cent. Anyone interested in this subject should read Foot's review and study Quensel's [18, 19] beautiful figures. Schlesinger's [22] study is also very useful.

In the vaginal smear the disadvantage of the sampling method is not apparent. There is usually a plethora of exfoliated cells since the whole epithelial surface of the genital tract may be represented. The diagnostic value of the smear depends on the greater tendency of malignant cells to be shed as compared to normal cells, as pointed out by Papanicolaou and Traut.[2] This loss of cohesiveness of the malignant cells has been measured by Coman,[23] who found that cells of carcinoma of the cervix could be separated with less than one-sixth of the force required to separate normal epithelial cells of the cervix. In spite of this there are usually relatively few carcinoma cells in a smear as compared to other cells, due to such factors as: (1) the ulceration, necrosis, and infection of the larger growths with decreased viability of the neoplastic cells, and also the masking of cells by the numerous polymorphonuclear leukocytes; (2) the greater fragility of malignant cells; (3) the small area of exposed tumor as compared to the whole surface of the genital tract. Variations in these factors explain why carcinoma cells may be

few or absent in the smears when large ulcerated growths are present, while appreciable numbers of well differentiated malignant cells may be scattered through the smear when the carcinoma is still *in situ* and not detectable on clinical examination.

Many points of practical importance in diagnosis of vaginal smears should be more explicitly demonstrated from detailed data on individual cases:

(a) What is the correspondence of a smear with (1) simultaneous clinical observation, and (2) simultaneous biopsy?

(b) In how many cases does a single smear suffice for the diagnosis?

(c) To what degree are consecutive smears consistent?

(d) How time-consuming is the examination of the smear?

(e) Can precancerous conditions be distinguished from cancer?

(f) How often is subclinical carcinoma, either as a primary condition or as recurrence after operation or radiation, detected by the smear?

(g) Is extensive cytologic experience necessary for gaining proficiency in diagnosis?

This present study is based on vaginal smears from patients of the out-patient gynecologic clinic of the New England Deaconess Hospital, from patients of the Pondville Hospital, of Westfield Sanatorium, of the Lahey Clinic, and from private patients of physicians using the State Tumor Diagnosis Service. We are indebted to Dr. J. V. Meigs, Chief of the Gynecological Clinic of the Palmer Memorial unit of the New England Deaconess Hospital, for the majority of the smears. The patients were in a sense selected in that the smear was not a routine procedure for all the patients in any of the clinics. Smears were made from some patients with no evidence of pelvic disease.

During the period of 9½ months from February through November 15, 1944, we examined 341 smears from 233 patients. The diagnosis of these smears was undertaken without any preliminary study and formed a small fraction of the routine work of the laboratories so that prolonged study of individual smears for diagnosis was not feasible. All reports were made without knowledge of the patients' histories or of the clinical diagnoses. Not until some time had passed did we attempt to study the group as a whole, and correlate the diagnoses on the smears with the clinical and pathologic findings. For this report, we have reviewed all of the smears.

The stain recommended by Papanicolaou and Traut [2] was used for nearly all of the smears.* The others were stained with hematoxylin and eosin and were quite satisfactory. One of the most important

* This stain was developed by G. N. Papanicolaou. (A new procedure for staining vaginal smears. *Science*, 1942, 95, 438–439.)

factors is the thinness of the smear. Too often only a small part of the secretions can be examined because of the density of the material. This can be easily overcome by spreading the material on more than one slide in a manner similar to making blood smears.

We shall review briefly some of the salient histologic features of the smears and lastly present our cases.

The normal and pathologic cells found in smears have been clearly described and beautifully illustrated in the monograph of Papanicolaou and Traut.[2] We shall, therefore, review only those histologic features of the smears which we have found of special importance. The most numerous normal cells are the squamous cells of the exocervix and vagina which vary in appearance according to their derivation from the inner (basal) or outer (superficial) part of the epithelium. The superficial cells are large, tend to be polyhedral, and the nuclei are very small in proportion to the cytoplasm. The "basal" cells are smaller, rounded, with a slightly larger nucleus than the superficial cell. Cells of both types undergo minor changes in structure under varying physiologic conditions, such as menstruation, pregnancy, and the menopause. While it is useful to understand these variations, an intimate knowledge of them is not essential for the study of malignancy, as none of them would cause any difficulty in differentiation from malignant cells. On the other hand, atrophy or infection of the cervix and vagina may produce pathologic changes of the "basal" cells closely simulating malignancy. Endometrial and endocervical cells are not commonly seen except during menstruation, when they occur singly and in clumps. They are minute in comparison with squamous cells, are oval, oblong, or pointed. The nucleus is finely powdered and the cytoplasm appears as a faintly colored fringe or tail at either end.

The various forms of malignant cells are too numerous to be described. A close study of the figures in the monograph by Papanicolaou and Traut[2] is most useful, especially after some experience with the smears. As would be expected, we have found nuclear changes to be the most reliable criteria of malignancy. A nucleus which is larger than normal in proportion to its cytoplasm should arouse suspicion, and still more should a relatively large nucleus with unusual appearance or arrangement of chromatin. The nucleus of the malignant cells we have seen in smears may be pale and finely powdered with granules; occasionally the chromatin is coarse, scattered, sometimes closely attached to the nuclear membrane. The nucleoli are often large, but stray granular fragments overlying the nucleus may suggest an enlarged nucleolus. In some malignant cells the nucleus is agranular and takes a homogeneous deep basophilic stain, but these cells usually

show some evidence of degeneration. In many of the malignant cells the centriole is distinctly visible. Mitotic figures are so seldom found that their significance is negligible. We have seen mitotic figures in smears in which the cells were atypical but not clearly malignant. Naked nuclei are frequent and often confusing but should not be taken seriously unless they are distinctly abnormal in structure. The staining reaction of the cytoplasm of the malignant cells is variable and has no practical significance. Striking variation in size of malignant cells has been found in relatively few cases and this characteristic may not be of great value inasmuch as nonmalignant normal and pathological cells are so conspicuously variable in this respect. However, as Papanicolaou points out, within a group of cells anisocytosis is quite significant. Variation in size may be greater in epidermoid carcinoma of low or moderate malignancy than in the less differentiated forms.[2]

The arrangement of carcinoma cells in clumps of more than three or four has not been a conspicuous feature in our preparations, or perhaps it would be more correct to say that we have had difficulty in evaluating the large sheets of cells which sometimes appear in smears. The masses are frequently so dense that no clear conception of the individual cells is possible, and the variation in size of these clumped cells is difficult to gauge. Papanicolaou and Traut [2] pointed out that groups of epithelial cells containing polymorphonuclear leukocytes are rarely found in nonmalignant conditions and should arouse suspicion of carcinoma, although this is not an absolutely pathognomonic change. In our experience, sheets of cells have been seen most often in cervicitis, vaginitis, and radiation reaction.

Single malignant cells do not always carry distinctive features indicating their origin. Those seen in smears often bear little resemblance to the normal cell types of the uterus seen in section, and the classification of the type of carcinoma is more difficult than the recognition of malignant cells. Papanicolaou and Traut [2] stated that "The grade I and II squamous carcinomas of the cervix . . . offer the least difficulty . . . although they are capable of producing a much greater variety of morphological variation," whereas, in the grade III squamous carcinomas many of the cells are less readily recognized as malignant and there are fewer malignant cells present. They also stated that many of the undifferentiated squamous carcinomas may produce cells difficult to distinguish from undifferentiated adenocarcinomas of the cervix. In differentiated carcinoma of the endometrium, the cells may not vary greatly from normal cells, but the presence of endometrial cells, except during menstruation, is abnormal and an unusual number or clumping of endometrial cells is strong evidence of adenocarcinoma.

However, in sections, the cells of an adenocarcinoma may not vary appreciably from those of hyperplasia. In smears, many of the cells are vacuolated with eccentric nuclei of signet ring type. In undifferentiated adenocarcinoma the cells may be very small with dark basophilic nuclei and only fine tails of cytoplasm.

The main points of our results will be found in the accompanying tables. Since it is our aim to evaluate the smear as a routine diagnostic procedure, we have given in each case the initial as well as the final diagnosis. The last diagnosis represents judgment based on our total experience to date and like the first was made without knowledge of the clinical or pathologic findings. Many of the smears were technically unsuitable. Some smears were entirely unsatisfactory and many others

TABLE I

General Summary of Cases

	Total	Total nonmalignant	Malignant†			
			Total malignant	Nonradiated	Radiated	
					Less than 3 months	More than 3 months
Cases	233	101	132	33	43	56
Smears*	341	123	218	45	75	98

* The numbers indicate smears taken at different times. Duplicate smears taken at the same time are counted as a single smear.

† In all but 4 of our cases classified as malignant there were positive biopsies, and clinically these 4 were unquestionably carcinoma.

nearly so. We graded the smears as good, fair, poor, or unsatisfactory (one-fourth of the smears were poor, and 16 others were unsatisfactory for diagnosis and were recorded as negative for statistical purposes). Since we wish to present our experiences entirely unbiased, we concluded that the fairest picture would be obtained by including all of the smears in our tables, although better smears would undoubtedly have eliminated some discrepancies. In two instances positive smears represented material aspirated directly from carcinomatous ulcers rather than from secretions.

In Table I the sum of our material is outlined. All but 4 of our cases classified as malignant had been determined to be positive by biopsy at one time or another. These 4 patients had gross tumors of unquestioned malignancy. Only 33 of the malignant cases (45 smears) were not radiated. The post-radiation cases are grouped as recent or remote depending on whether there had been a biopsy within 6 months of the time the smear was taken. The 86 recent cases (159 smears) were mainly carcinoma of the cervix treated by radiation. Thirteen

of these were recurrent carcinoma of the cervix 1 to 4 years after radiation. Sixteen of the recent cases (31 smears) were of adenocarcinoma or adenoacanthoma. The 46 remote cases (59 smears) had been examined by biopsy anywhere from 7 months to 11 years before the smear was taken. These 46 patients, with one exception, had been radiated. Twenty-one of them had remained without evidence of disease for 2 to 11 years.

The types of lesions of malignant and nonmalignant cases are shown in Table II. The cases classified as nonmalignant were considered clinically as benign, but only 36 of them were examined by biopsy. We have arbitrarily included in this group several exceptional cases: 3 patients who had had a hysterectomy for adenocarcinoma of the fundus 2 to 4 years before the smear was taken and since had been free from disease; 1 case with localized carcinoma of the vulva; 9 cases with incomplete data; 6 cases (9 smears) without any clinical data.

Table III shows the distribution of the revised diagnoses on the radiated and nonradiated group. The smears in which the first and final diagnosis did not correspond exactly are here tabulated in sequences of 50, to show the effect of experience. There are 10 other smears not tabulated, 9 of them from radiated patients, which we have not been able to interpret. Although there are nearly the same number of smears in the nonradiated and radiated groups (168 to 173), the majority of the changes in diagnosis were on smears from radiated patients. These smears with a change in diagnosis on the final review are given again with the complete data on the patients in the three following tables.

In Tables IV, V, and VI are cases in which the diagnoses from the smears did not closely correspond with the other data. These cases are grouped as follows:

Table IV—Clinically benign and doubtful cases

Table V—Malignant cases not radiated

Table VI—Malignant cases radiated

The clinical and pathologic diagnoses are those made *at the time* the smear was taken or within a week or 10 days before the smear was taken, provided no treatment intervened. The column headed "Type of Carcinoma" is included for the benefit of those smears without a simultaneous biopsy and gives the pathologic diagnosis made at an earlier or later date. All but a few of the pathologic diagnoses were made at another laboratory. We have chosen this rather elaborate form of presentation of cases as the clearest way to correlate all of the information on a single patient, and to show the type of case in which doubt and error are most likely to occur. These three tables present

TABLE II
Types of Lesions

	Benign					Malignant								Grand total
	Cervicitis or vaginitis		Other		Total benign	Epidermoid carcinoma				Total	Adeno-carcinoma	Other‡	Total malignant	
	Biopsy	No biopsy	Biopsy	No biopsy*		I	II	III	Not graded†					
Cases	18	5	13	65	101	8	23	33	29	93	19	20	132	233
Smears	23	5	18	77	123	17	36	51	49	153	35	30	218	341

* This includes incomplete cases.
† Undifferentiated carcinoma and carcinoma insufficient for classification.
‡ Includes 4 unbiopsied cases.

every case in which there is any inconsistency in the data. The discrepancies in these tables are of two kinds: (1) Changes in the diagnoses of the smears. This is an elaboration of the data in Table III. (2) Discrepancies between the final diagnosis on the smear and the clinical and pathologic diagnoses. (a) Some of these differences are probably due to errors of interpretation, especially to failure to recognize the malignant cells, but definite proof of error is lacking. (b) In a few instances the patient was clinically free of disease but biopsy and smear proved its presence.

In the group of 123 smears from 101 patients without clinical or pathologic evidence of malignant disease in the genital tract, there is one false positive diagnosis, and this an initial error. Table IV includes this case with 8 other doubtful cases. The definite false positive smear is no. 726, case 30. This was taken from a woman, 82 years old. Three biopsies showed chronic cervicitis and 3 subsequent smears were called negative. Our final diagnosis on this smear is negative.

Table V presents 12 malignant, nonradiated cases lacking conformity in diagnoses of smear and clinical and pathologic data. Eight cases are included because of a doubtful initial diagnosis or a final change in diagnosis. One case, no. 96, is also in Table VI since all but the first smear were taken during radiation. Cases 18 and 31 will be discussed later in connection with early carcinoma.

TABLE III

Changes in Interpretation of Smears Arranged in Chronologic Series of 50 Smears Each

Changed from positive to negative	1st	Radiated	2nd	Radiated	3rd	Radiated	4th	Radiated	5th	Radiated	6th	Radiated	Total not radiated	Total radiated	Grand Total
Not radiated	1														
During radiation															
1 to 6 mos. post-radiation		1													
6 mos. to yrs. post-radiation		3		1											
Totals													1	5	6
Changed from negative to positive															
Not radiated									1						
During radiation				2		1		1				1			
1 to 6 mos. post-radiation						1		1		1					
6 mos. to yrs. post-radiation				1		1		1		1		1			
Totals													1	13	14

TABLE III (*Continued*)

Changed from questionable to negative	1st	Radiated	2nd	Radiated	3rd	Radiated	4th	Radiated	5th	Radiated	6th	Radiated	Total not radiated	Total radiated	Grand Total
Not radiated	1		1		1				1						
During radiation						1									
1 to 6 mos. post-radiation		2						1							
6 mos. to yrs. post-radiation		4		2		1		1		1					
Totals													4	13	17
Changed from questionable to positive															
Not radiated	1		1		1		1		3						
During radiation				2		2						2			
1 to 6 mos. post-radiation		2										1			
6 mos. to yrs. post-radiation															
Totals													7	9	16

The cases which were radiated are more difficult to analyze. In many of them the interval between the biopsy and the smear was too long for correlation. Smears taken during radiation were frequently unsatisfactory because of degeneration. The greatest difficulty, however, arises from an uncertainty of interpretation of the cases in which radiation reaction is present. In Table VI are those radiated cases in

TABLE IV

Cases Which Were Not Malignant from Clinical or Pathologic Evidence in Which Smear Diagnosis Did Not Conform to Other Data

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data	
					Clinical diagnosis	Diagnosis by biopsy
30	726	+	−		Cervicitis	Cervicitis
	892	−	−	12 days		
	1157	−	−	3 weeks		
	2437	−	−	4 months		
47	1002	Suggests low-grade carcinoma; not definite	−		Negative	
	1159	−	−	2 weeks		
86	1737	Cells suggesting tumor present	−		Chronic cervicitis	Chronic cervicitis
	2041	−	−	3 weeks		
149	2599	+(?)	−		Cervicitis	Cervicitis
189	3033	−	−		Endometriosis	
	3371	+	+	1 month	Endometriosis	Early epidermoid carcinoma(?)
202	3196	+	+		Carcinoma of cervix(?)	Negative
206	3200	+	+		Uncertain	Negative
214	3208	Scattered carcinoma cells	+		Pelvic tumor	Negative
229	3370	+	+		Uncertain	Hyperplasia of endometrium

which the first or last diagnosis on the smear differed from the other data. These discrepancies are not necessarily errors of the method, as study of the table will show. They represent also deficiencies in the biopsy procedures and in clinical data.

Thus far we have considered the smear chiefly as subordinate evidence subject to correction or confirmation by biopsy. In some cases the positive smear may be the first, or even the only, preoperative evidence of carcinoma, and it is in lesions of this sort that the smear may have the greatest importance as an accessory method of diagnosis.

TABLE V

Discrepancies Between Smear and Other Diagnoses on Malignant, Not Radiated, Cases

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data		
					Clinical diagnosis	Biopsy diagnosis	Type of carcinoma
18	458	−	−		Negative	Very early carcinoma	Very early carcinoma
	1217	−	−	2 mos.			
	1865	−	−	2½ mos.			
	2762	−	−	3 mos.			
	3538	−	−	2 mos.			
27	627	One cell suggests carcinoma	−		Uncertain	Undifferentiated carcinoma, endometrium	Undifferentiated carcinoma, endometrium
28	628	One cell suggests carcinoma	+		Carcinoma	Carcinoma	Epidermoid carcinoma, grade III
31	728	+	+		Negative	Carcinoma *in situ*	Carcinoma *in situ*
50	1079	One carcinoma cell(?)	+		Carcinoma	Carcinoma	Epidermoid carcinoma, grade III
	1139	+	+	1 week	Carcinoma		
	3452	−	−	7 mos.	Carcinoma	Epidermoid carcinoma, grade III	
81	1693	−	+(?)		Carcinoma	Epidermoid carcinoma, grade I	Epidermoid carcinoma, grade I
	2331*	−	−	2 mos.			

TABLE V (*Continued*)

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data		
					Clinical diagnosis	Biopsy diagnosis	Type of carcinoma
96	2040	−	+		Senile vaginitis	Carcinoma	Adenocarcinoma
	2296	−	+	3 weeks			
	2485	+	+	3 weeks			
	3142	−	−	2 months		Carcinoma	
103	2187	Suggests carcinoma(?)	+		Carcinoma	Carcinoma	Adenocarcinoma
	3019	Suggests carcinoma(?)	+	2½ months			
146	2587	Few carcinoma cells(?)	+		Carcinoma	Epidermoid carcinoma	Epidermoid carcinoma, grade II
173	2842	−	+		Carcinoma	Adenocarcinoma	Adenocarcinoma
	3076*	+	+	3 weeks			
174	2869	Probably adenocarcinoma	+		Senile vaginitis	Carcinoma	Adenocarcinoma
220	3254	−	−		Stage 3 lesion	Epidermoid carcinoma, grade III(?)	Epidermoid carcinoma, grade III

* These smears were taken during, or immediately after, radiation.

TABLE VI

Discrepancies Between Diagnoses from Smear and Other Diagnoses on Cases of Post-Radiation Carcinoma

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data			Radiation reaction	Type of carcinoma
					Simultaneous clinical diagnosis	Simultaneous biopsy diagnosis	Time between radiation and smears		
1	171	−	−		Carcinoma	Epidermoid carcinoma, grade II	1 month	−	Epidermoid carcinoma, grade II
	893	−	+(?)	2 months	Uncertain		3 months	−	
	1866	−	−	3 months	Uncertain		6 months	−	
	2841	+	+	3 months	Carcinoma		9 months	+	
5	209	A few carcinoma cells	−		No tumor		7 years	+	Adenoacanthoma
	465	Two carcinoma cells	−	3 weeks				+	
	727	A few small carcinoma cells	−	3 weeks				+	
	1445	−	−	2 months				+	
	2439	−	−	3 months				+	
6	210	Rare cells suggesting carcinoma	−		Negative		4 months	+	Adenocarcinoma
	461	Carcinoma cells(?)	−	3 weeks			5 months	+	
	1297	Probably carcinoma	?	2½ months			8 months	+	
	2274	−	−	3 months			11 months	+	
10	296	−	−		Carcinoma		9 months	?	Adenocarcinoma
	462	+	+	2 weeks			9 months	?	
	1158	Suggests carcinoma(?)	+	2 months		Adenocarcinoma	11 months	−	
	2196	+	+	3½ months	Carcinoma		14 months	−	
	3257	+	+	3 months	Carcinoma		2 months	−	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
11	297	+	+		Negative	Negative	8 months	+	Carcinoma
12	352	Carcinoma(?)	+		Carcinoma	Carcinoma	During radiation	?	Adenocarcinoma
	806	Carcinoma(?)	+	5 weeks			At end of radiation	−	
17	370	A few cells suggesting well differentiated carcinoma	−		Negative		3 years	+	Epidermoid carcinoma, grade III
21	463	+	+				4 years	?	Epidermoid carcinoma, grade III
	1078	One malignant cell(?)	+	2 months		Carcinoma with radiation reaction	During radiation	?	
	1583	−	+(?)	2 months		Rare cells suggesting carcinoma	3 months	?	
	2426	−	+	3 months	Negative	Radiation reaction with a few carcinoma cells	5 months	+	
22	464	Sheets of carcinoma cells	−		Abdominal mass		1 year	+	Adenocarcinoma
	808	A few cells suggest carcinoma	−	1 month	No vaginal or uterine tumor		13 months	?	
24	467	−	−		Carcinoma		2 months	−	Epidermoid carcinoma, grade III

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data			Radiation reaction	Type of carcinoma
					Simultaneous clinical diagnosis	Simultaneous biopsy diagnosis	Time between radiation and smears		
26	626	Scattered cells suggesting well differentiated carcinoma	–		Negative		2 years	?	Epidermoid carcinoma, grade I
	1577	–	–	3 months	Negative		2 years	?	
	2560	A few cells suggest carcinoma	–	3 months	Negative			+	
34	809	+	+		Carcinoma		4 years	+	Epidermoid carcinoma, grade III
	2484	–	+	5 months	Extensive disease			+	
35	810	+	+		Negative		8 months	+	Epidermoid carcinoma, grade III
	3079	Atypical cells; no definite carcinoma	+	7 months	Ulcer, radiation reaction(?), carcinoma(?)	–	2 months	+	
38	894	Low grade carcinoma	–		Negative		3 months	+	Epidermoid carcinoma, grade II
40	896	–	+		Carcinoma	Epidermoid carcinoma, grade II		–	Epidermoid carcinoma, grade II
	1172	–	+	3 weeks	Carcinoma	Epidermoid carcinoma, grade II	At time of radiation	?	
	1579	–	–	6 weeks	Carcinoma		1 month	–	
	2909	–	–	4 months	Carcinoma	Wertheim's operation; negative	5 months	–	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
46	1001	Low grade carcinoma	–		Negative		5 years	+	Epidermoid carcinoma, grade I
	1137	–	–	2 weeks				+	
	1215	–	–	1 week				?	
	2189	–	–	3 months				–	
53	1140	Suggestive carcinoma cells	–		Disease present		2 months	–	Epidermoid carcinoma, grade II
	2190	–	–	3 months			5 months	–	
56	1213	–	–		Negative		4 months	–	Epidermoid carcinoma, grade I
	2195	A few cells suggesting carcinoma, not definite	–	3 months			7 months	–	
	2840	–	–	2 months			8 months	+	
58	1216	–	–		Extensive disease		4 years	+	Epidermoid carcinoma
	3530	Atypical cells degenerative, radiation reaction(?), carcinoma(?)	+(?)	7 months	Negative	–	2 months		
60	1219	Suggests carcinoma	–				2 years	+	Carcinoma of cervix
	2191	–	–	3 months				+	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data			Radiation reaction	Type of carcinoma
					Simultaneous clinical diagnosis	Simultaneous biopsy diagnosis	Time between radiation and smears		
65	1359	−	+		Carcinoma	Epidermoid carcinoma, grade III	5 months	?	Epidermoid carcinoma, grade III
	2188	+	+	2½ months		Radiation reaction with carcinoma	At completion of radiation	−	
69	1363	Low grade carcinoma(?)	−		Negative		1 year	−	Epidermoid carcinoma
	2987	−	−	5 months			5 months	−	
70	1425	−	−		Carcinoma			−	Unknown
	1466	−	−	4 days			During radiation	−	
76	1535	−	+		Carcinoma	Epidermoid carcinoma ungraded with radiation reaction of stroma	2 months	−	Epidermoid carcinoma
	1582	2 cells suggest carcinoma	?	1 week	Carcinoma of cervix	Epidermoid carcinoma with radiation reaction of stroma	2 months	?	
82	1694	Suggests carcinoma	−		Negative		8 years	+	Epidermoid carcinoma, grade III
85	1736	Suggests carcinoma	−		Carcinoma of vagina	Epidermoid carcinoma, grade I	During radiation	−	Epidermoid carcinoma, grade I
	1739	Suggests carcinoma	+	Simultaneous	Carcinoma		During radiation	−	
	1811	−	−	3 days	Carcinoma		During radiation	−	
	1902	−	−	1 week	Carcinoma		During radiation	−	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
94	1966	−	+(?)		Recurrence(?)		13 months	−	Epidermoid carcinoma, grade III
96	2040	−	+		Senile vaginitis	Carcinoma		−	Adenocarcinoma
	2296	−	+	3 weeks			During radiation	−	
	2485	+	+	2 weeks			1 month	+	
	3142	−	−	2 months		Carcinoma	3 months	−	
103	2187	Suggests carcinoma(?)	+		Carcinoma	Carcinoma	Before radiation		Adenocarcinoma
	3019	Suggests carcinoma(?)	+	2½ months			At end of radiation	+	
107	2222	+	+					−	Epidermoid carcinoma
	2241	−	−	2 days		Epidermoid carcinoma with radiation reaction	After radiation	−	
109	2299	−	−		Carcinoma	Radiation reaction with small focus of carcinoma	During radiation	−	Carcinoma
	3342	+(?)	+(?)	3 months	Carcinoma	−	3 months	−	
122	2413	−	+		Negative	Carcinoma	2 years	−	Epidermoid carcinoma, grade III
130	2422	+	+		Negative	Negative	4 months	−	Adenoacanthoma
142	2472	Probably carcinoma(?)	+			Carcinoma	During radiation		Epidermoid carcinoma, grade III

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data			Radiation reaction	Type of carcinoma
					Simultaneous clinical diagnosis	Simultaneous biopsy diagnosis	Time between radiation and smears		
145	2564	−	+		Uncertain		4 months	−	Epidermoid carcinoma
	2699	+	+	1 week		Radiation reaction with two carcinoma cells	5 months	−	
169	2759	+	+		Extensive carcinoma of stump	Carcinoma unclassified			Epidermoid carcinoma, grade III
	3301	A few cells suggesting well differentiated carcinoma	+(?)	1½ months		Epidermoid carcinoma, grade III	During radiation	−	
171	2763	+	+		Negative		1 year	+	Epidermoid carcinoma
180	2940	Suggests carcinoma	+		Uncertain	Necrotic tissue(?)	2 months	−	Epidermoid carcinoma
188	3032	+	+		Negative		4 months	−	Epidermoid carcinoma
191	3035	Atypical carcinoma cells(?)	Radiation reaction(?), tumor(?)		Negative		2 years		Epidermoid carcinoma, grade III
193	3037	−	+		Carcinoma	Adenocarcinoma	4 months	−	Adenocarcinoma
197	3141	3 cells very suggestive of carcinoma	?		Negative		3 years		Epidermoid carcinoma, ungraded

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data			Radiation reaction	Type of carcinoma
					Simultaneous clinical diagnosis	Simultaneous biopsy diagnosis	Time between radiation and smears		
200	3145	Adenocarcinoma(?)	+		Carcinoma	Carcinoma with radiation reaction	During radiation	−	Epidermoid carcinoma
	3229	+	+	5 days		Carcinoma with radiation reaction	During radiation	−	
209	3203	−(?)	−(?)		Carcinoma		7 months	+	Epidermoid carcinoma, grade I
213	3207	+	+		Recurrent carcinoma(?)	No evidence of tumor	2 years	−	Not specified
228	3349	Cells poorly preserved, suggesting carcinoma	+(?)		Radiation reaction	−	10½ months	−	Papillary adenocarcinoma of ovary
235	3531	+	+		No tumor		10 months		Epidermoid carcinoma, grade III

There are four cases in Tables IV, V, and VI in which a positive diagnosis on the smear was the first evidence of carcinoma: a case of carcinoma *in situ;* 3 cases of "hidden" or unsuspected carcinoma, including an adenocarcinoma and one post-radiation recurrence. More weighty evidence than our 4 cases has been given by others with regard to "hidden carcinoma" and carcinoma *in situ.* Papanicolaou and Traut [2] reported that among the 193 cases of malignant tumors of the genital tract diagnosed by the vaginal smear, 9 were unsuspected clinically. Meigs *et al.*[3] reported 3 cases possibly of this sort but still unproved when reported. Carcinoma *in situ* was reported by Papanicolaou and Traut in 7 of 127 carcinomas of the cervix, 100 being squamous-celled carcinoma. This is an exceptionally high proportion of cases. Meigs *et al.* reported 10 (22 per cent) clinically early carcinomas of the cervix and 5 early cases of carcinoma of the endometrium. The diagnosis was confirmed in each of these 15 cases.* The only early carcinoma in our series, excepting the carcinoma *in situ* (case 31, Table V), was an unexpected finding after hysterectomy (case 18, Table V). This was a completely keratinized, well differentiated, slightly invasive carcinoma. It did not produce cells in the smear recognizable as malignant.

We wish to emphasize a third condition—recurrence after radiation—in which the smear may be invaluable in demonstrating carcinoma. In such cases there may be a real hesitancy in performing a biopsy. In our material there are 13 cases of recurrent carcinoma proved by biopsy 1 to 4 years after radiation. The recurrences under 1 year are not considered in this connection because of the difficulty of distinguishing between persistence, radiation necrosis of carcinoma, and recurrence. In only 1 of these 13 cases did the smear give the first indication of recurrence. Early recurrent carcinoma may be very difficult to recognize due to radiation reaction.

Radiation reaction as seen in sections of tissue is recognized not by a single specific change but by a concurrence of changes in stroma, blood vessels, and epithelial cells. Anaplasia, often prominent in radiation reaction, is readily distinguished from neoplasia in sections but not so in smears. The isolated radiated cells may closely resemble malignant cells. There are two prominent features of smears showing radiation reaction: a high proportion of "basal" cells and variation in their appearance. These "basal" cells are large with swollen nuclei, which tend to be pale rather than hyperchromatic or very small with

* Jones *et al.* reported three unsuspected instances in patients attending an endocrine clinic. (Jones, C. A., Neustaedter, T., and Mackenzie, L. L. The value of vaginal smears in the diagnosis of early malignancy. *Am. J. Obst. & Gynec.*, 1945, **49**, 159–168.)

deeply pyknotic nuclei. There is usually variation in shape as well as in size. Cells of undetermined type may represent injured endocervical epithelium (Fig. 3). Obvious signs of degeneration are seen in some of the cells as, for example, the heavily spotted nuclei, with large distinct clumps of chromatin sometimes without a nuclear membrane (Fig. 5). Such nuclei may be seen in cervicitis and are identical with nuclei in some early epidermoid carcinomas (Figs. 33 and 39 of Plate E [2]), and others resemble Figures 11 and 27 in that plate. Many of the radiated cells closely resemble the carcinoma cells of a grade I epidermoid carcinoma depicted in Figure 6, Plate J.[2] Some of these very atypical cells are seen in patients who have remained without evidence of disease 5 or more years after radiation treatment. This similarity of the radiated and malignant cell is well brought out by our Figures 3 and 4, which represent radiated epithelium of endocervical glands. The preparation was made from a uterus removed after radiation for epidermoid carcinoma, grade I, of the cervix. There was no residual tumor found in multiple sections but there was marked radiation reaction of the epithelium of the endocervix.

Some of the cell changes prominent in radiation reaction may also be seen in nonirradiated elderly patients, more often in the seventh and eighth decades than earlier, and usually are associated with infection. The differentiation of radiation reaction from changes due to atrophy and infection on the one hand and from malignant cells on the other depends on the cell picture as a whole rather than on the appearance of certain cell types. Thus, in radiation reaction there are more cells that differ from one another in appearance and usually more anaplastic cells than are seen in nonradiated benign conditions. We have reviewed all of our smears in the series without reference to other data, for the purpose of determining the presence of radiation reaction. The extent of radiation reaction is unpredictable and cannot be correlated with the time element or the amount of radiation. Only 46 of 157 smears from radiated patients showed definite radiation reaction. Twenty-three others were suggestive of radiation reaction. There were 10 smears which we thought showed radiation reaction but the patients had not been radiated. Four of the patients were over 70 years; 4 were between 60 and 70; 2 were 47 and 56 years old. In 3 of the cases carcinoma was present in the smears; the others showed cervicitis.

We cannot show as clear-cut results with the smears as others have reported. We have found "accuracy" a thing not easily determined as applied to diagnosis of smears. The fact that the diagnosis confirms other data does not necessarily attest to its correctness. Thus in a case reported by Meigs *et al.*, in which the vaginal smear was positive, the

initial pathologic diagnosis on the removed uterus was negative for tumor, but multiple sections demonstrated a minute focus of tumor in 2 of 50 slides.[3]

Our report leaves unanswered the all-important question in respect to the consistency with which the smear represents the condition of the genital tract. It is the general impression of those using the method that if carcinoma is present the cells may be found fairly consistently in the smears. Meigs *et al.* made the statement that of 16 successive smears taken from a woman known to have cancer, in a period of 7 days, only 6 smears contained cancer cells.[3] This statement alone would count heavily against the practicability of the method. It does demonstrate that the diagnosis of cases by multiple smears is more accurate than diagnosis based on single smears. The problem of accuracy of interpretation of the smear and the problem of the representative character of the smear have each to be considered. Since thus far no data have been presented on the accuracy and consistency of each smear taken on a given case, we have given in detail our interpretation of each smear submitted, based on pathologic criteria alone, and have correlated this with other data of the case. The high proportion of radiated cases in this group and the limited experience afforded by the small sample permit only limited conclusions as to the general usefulness of the method.

Summary and Conclusions

A total of 341 vaginal smears from 233 women were studied to determine the place of this method in routine diagnosis of the presence or absence of uterine carcinoma.

A mistaken positive diagnosis of a negative smear should be a problem only in exceptional cases of radiation reaction or of senile atrophy with infection.

False negative diagnoses are more difficult to avoid. There are certain types of carcinoma cells which are difficult to recognize without a good deal of experience.

Interpretation of smears made after radiation treatment demands more experience than other smears because of the changes in epithelium induced by radiation.

The smear may be valuable in diagnosis to detect recurrence after radiation since in these cases biopsy is preferably avoided.

As a subsidiary test, the vaginal smear may be especially useful in cases of "hidden carcinoma."

A pathologist will not need special experience to recognize untreated carcinoma of the cervix of low to moderate malignancy, and a relatively

short period of training may suffice for a technician. On the other hand, smears from carcinoma of the cervix of high malignancy, sloughing tumors, some adenocarcinomas of the endometrium and endocervix, as well as irradiated carcinomas may be difficult problems for a pathologist even after some familiarity with the method.

The time taken in examination of the smear, always provided it is carefully prepared, may vary from 2 or 3 to 20 minutes. This limit is set from the standpoint of laboratory practice, not research. This method as a means of final diagnosis has yet to be clearly established. Before this is possible, there must be more specific information upon the limitations and advantages of the method based on larger series of cases. It is our impression that this procedure is promising in a high degree.

As a screening test for detecting the existence of cervical or endometrial cancer in large groups of women it may well be of value.

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DESCRIPTION OF PLATES

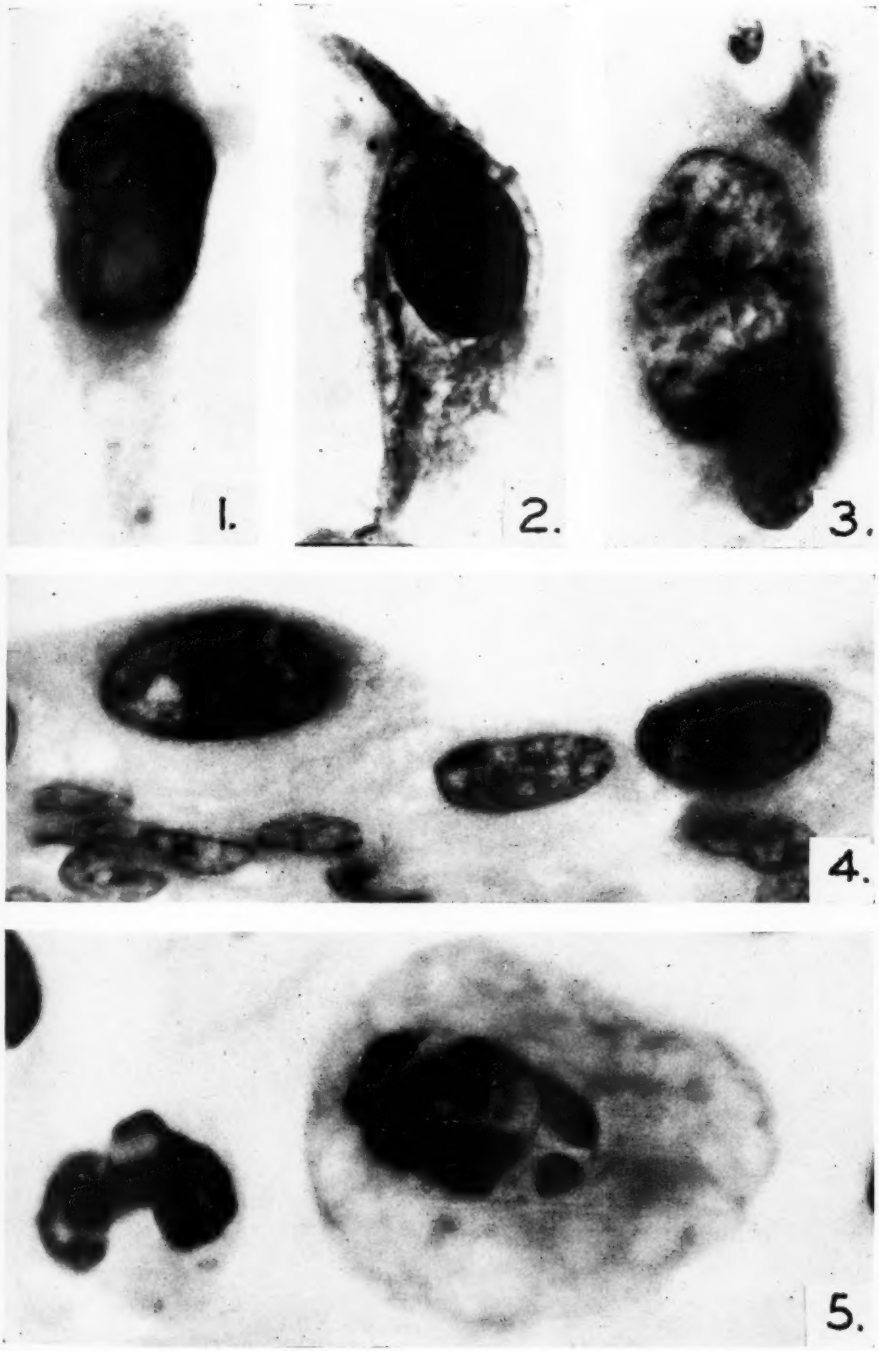
PLATE 96

FIG. 1. Malignant cell in vaginal smear from patient with epidermoid carcinoma, grade III, of cervix. × 2000.

FIG. 2. Malignant cell in vaginal smear from patient with carcinoma *in situ.* × 2000.

FIGS. 3 and 4. Endocervical epithelium showing radiation reaction. This is photographed from a section of the removed uterus. × 2000.

FIG. 5. Cell showing nuclear degeneration from vaginal smear of patient radiated for carcinoma. × 4000.



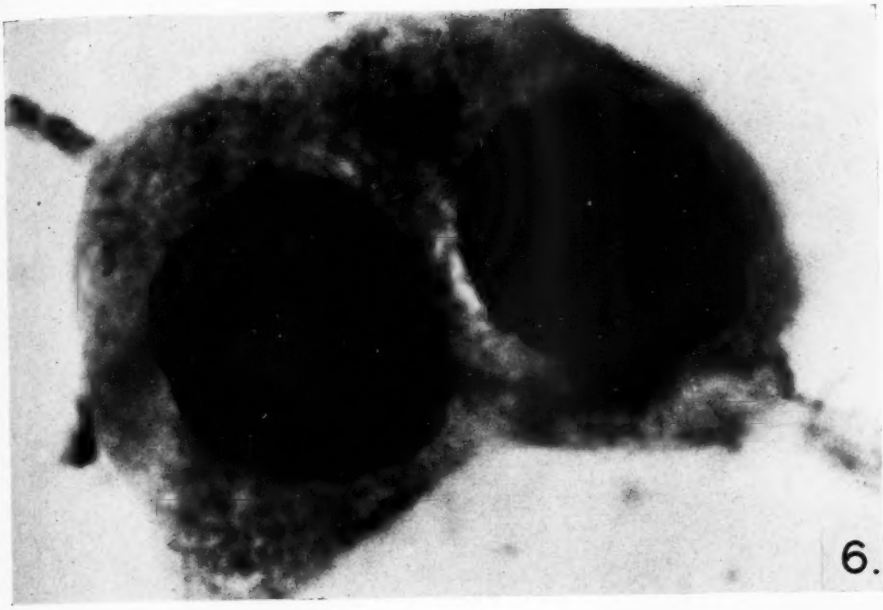
Gates and Warren Vaginal Smear in Diagnosis

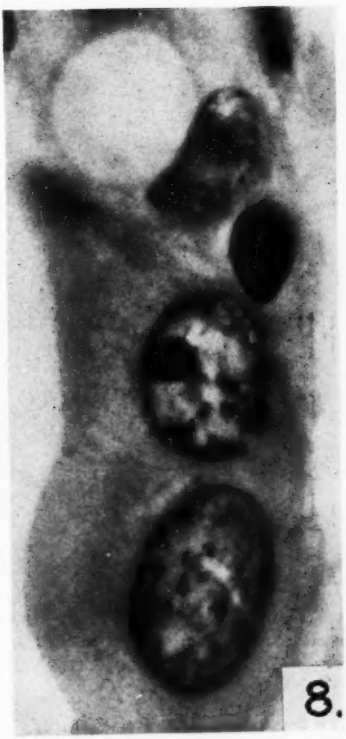
PLATE 97

FIG. 6. Two cells from smear of patient with carcinoma *in situ*. × 4000.

FIG. 7. Atypical endocervical epithelium showing results of radiation. Photograph of cells in section from removed uterus. × 4000.

FIG. 8. Endocervical epithelium from same case as Figure 7. × 2000.





Gates and Warren Vaginal Smear in Diagnosis

PLATE 98

These photographs are of cells in smears. × 2000.

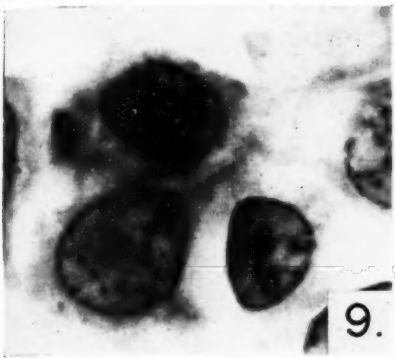
FIG. 9. Epidermoid carcinoma, grade III.

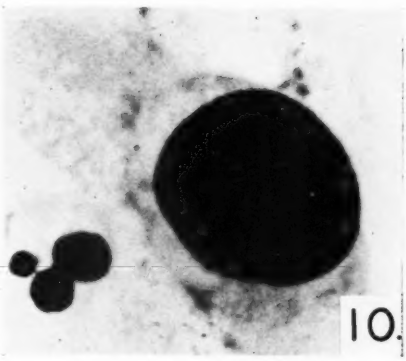
FIG. 10. Epidermoid carcinoma, ungraded.

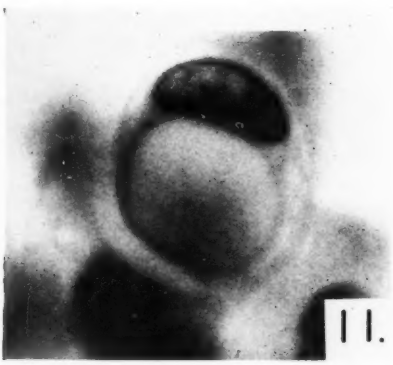
FIG. 11. Adenocarcinoma.

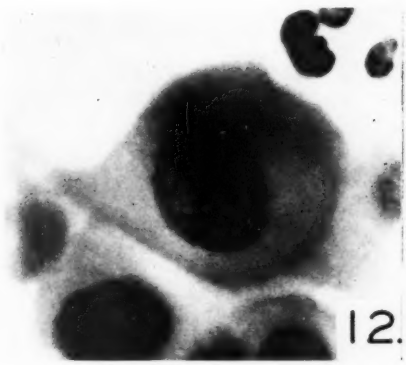
FIG. 12. Atypical basal cell.

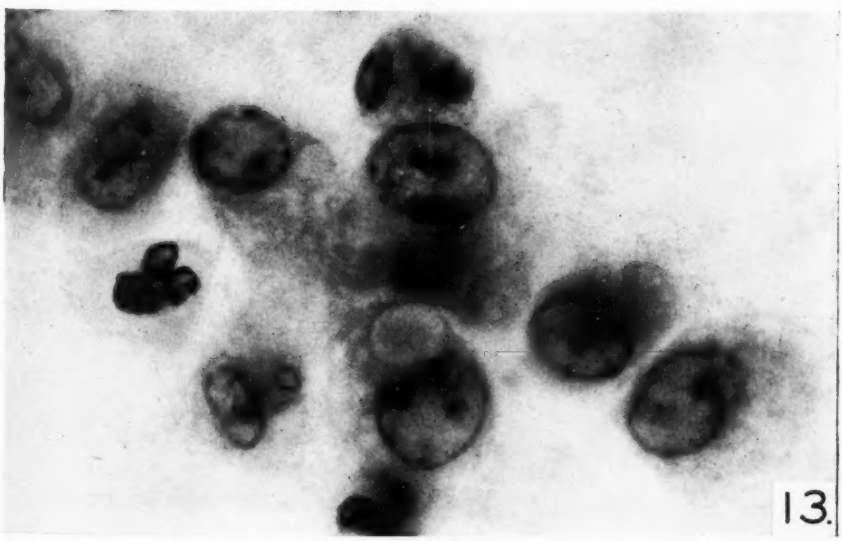
FIG. 13. Adenocarcinoma.











A COMPARATIVE STUDY OF THE PATHOLOGY OF SCRUB TYPHUS (TSUTSUGAMUSHI DISEASE) AND OTHER RICKETTSIAL DISEASES *

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During and immediately after the last war, literally millions of lives of both the civilian and military population of the Near East were sacrificed to the louse-borne typhus fever. In this war, although our troops have been exposed to endemic and epidemic foci, not a single American soldier has died of louse-borne typhus fever. This record, brought about in large part through the use of a consistently effective vaccine, surely constitutes one of the outstanding achievements in preventive medicine. Unfortunately, the situation with regard to scrub typhus is considerably different. To date, no reliable vaccine, antiserum, or chemotherapeutic agent has been devised to combat the disease which is precariously widespread throughout the Far East. It is therefore imperative that the facts as offered by all the pertinent sciences, including pathology, be collected and integrated into a unified workable account. Toward this end, there have been collected at the Army Institute of Pathology, through the efforts of the U. S. A. Typhus Commission † and of individual medical officers, 100 cases of scrub typhus of which the first 78 form the basis of this report, 24 cases of epidemic louse-borne typhus, and 12 cases of Rocky Mountain spotted fever.‡ In addition, through the kindness of Dr. R. D. Lillie, Senior Surgeon of the U.S. Public Health Service, the histologic slides of 2 cases of American "Q" fever were made available. It was our purpose not only to try to learn the lesions of scrub typhus, but to determine if there were any changes which were sufficiently characteristic and constant as to permit a histologic differentiation of the various typhus fevers.

NOSOLOGY

For purposes of practical nosologic orientation we have tabulated the rickettsioses as shown in Table I.

In this communication we are concerned primarily with the tsutsugamushi group which, like the spotted fever group, has been subdivided

* Received for publication, January 15, 1945.

† This material consists of pathologic specimens from 24 cases of louse-borne typhus fever observed in Egypt by Lt. Comdr. W. B. McAllister, M.C. (U.S.N.R.), member of the U.S.A. Typhus Commission. Of these cases, 23 were used as the basis for comparison in this study. Grateful acknowledgment is made to Lt. Comdr. McAllister for permission to use this material.

‡ The tissues of 72 cases of scrub typhus and of all the cases of Rocky Mountain spotted fever were fixed in formaldehyde solution; the tissues of the 6 remaining cases of scrub typhus and of all the epidemic typhus were fixed in Regaud's solution.

into various entities that were born chiefly of geographic barriers rather than real, inherent distinctions. While it is true that certain differences exist, such as great discrepancies in mortality rates, as well as the absence of a primary lesion or eschar in at least one form (Malayan),

TABLE I

Classification of the Rickettsioses

Disease	Vector	Weil-Felix Reaction †
I. Typhus Group		
A. Epidemic typhus (tabardillo)	Louse	OX_{19} +++,
Brill's disease *		OX_2 +,
B. Murine (endemic) typhus	Flea	OXK —
II. Spotted fever group		
A. Rocky Mountain spotted fever	Tick	OX_{19} +
B. São Paulo fever		OX_2 +
C. Colombian spotted fever		OXK +
D. Kenya typhus		
E. South African tick fever		
F. Fièvre boutonneuse		
III. Tsutsugamushi fever group	Larval mite	OX_{19} —
A. Tsutsugamushi (Japan, Formosa, etc.)		OX_2 —
B. Scrub typhus (New Guinea, etc.)		OXK +++
C. Queensland coastal fever		
D. Malayan (rural, tropical) typhus		
E. Sumatran mite typhus		
IV. Miscellaneous group		
A. "Q" fever		
1. Australian "Q" fever	Tick (?)	
2. American "Q" fever	Tick (?)	OX_{19} —
B. Trench fever	Louse	OX_2 —
C. Colorado tick fever ‡	Tick	OXK —
D. Texas tick fever ‡	Tick	

* Brill's disease is currently regarded as a recrudescence of latent louse-borne, epidemic typhus.

† The degrees of reaction as herein indicated are, of course, arbitrary. Variations may occur, particularly in the spotted fever group.

‡ Proof of the rickettsial etiology of these diseases is not yet universally accepted.

there is considerable immunologic evidence that these diseases are essentially the same.[1-3] The phenomenon of differences in reactions of different peoples to an organism, which itself may vary in habits, hardiness, and heritage, has many well known analogies in Medicine; to wit, the markedly varied response of certain nationalities and races to tuberculosis, spirochetoses, streptococcal infections, etc. The cases in the current series developed in New Guinea and the disease will hereafter be called "scrub typhus."

CLINICAL PICTURE

Detailed clinical, epidemiologic, and laboratory reports will be forthcoming from other sources. Therefore, only a brief summation of these

data will be included here. The etiologic agent is a rickettsia (*R. orientalis* or *R. tsutsugamushi*) of which the reservoir is probably the rat, field mouse, or bandicoot. The rickettsiae are transmitted to man by the larval forms of the mites, *Trombicula akamushi, T. deliensis, T. hirsti,* and others. The larval mites are encountered in the tall lalang or kunai grass, brush, or secondary jungle growth consisting of stunted trees or "scrub." The larval mite acquires rickettsiae by sucking a meal of blood from a vertebrate host. The organisms are transmitted through the ova to succeeding generations of mites. The adult mites do not feed on man. At the site of the bite, a characteristic primary lesion, called an eschar, develops. The host may not notice the lesion until the disease is clinically evident, or he may fail to notice it entirely. The incubation period varies from 10 to 18 days.[4] The onset is sudden and is characterized by chilliness or rigors, headache, and fever. A macular or slightly papular rash appears on the 5th to 8th day, usually fading in 4 or 5 days. The rash is to be distinguished from dengue, measles, dermatitis medicamentosa, etc. An enanthem may develop on the soft palate, but the buccal surface of the cheeks is spared. In severe cases, the rash may be petechial. The lymph nodes, especially those draining the site of the eschar, are enlarged and slightly tender. Cough occurs early and is troublesome. Pneumonia, both bronchopneumonia and the interstitial variety, is frequent. Injected conjunctivae and photophobia, as in other typhus fevers, are common. The headache and fever persist and may be accompanied by nausea, vomiting, diarrhea, epistaxis, apathy, transient deafness, stiffness of neck, clouding of sensorium, and often querulousness, insomnia, delirium, convulsions, pareses and paresthesias, and disturbances of superficial and deep reflexes. In severe cases, the pulse is rapid; hypotension, shock, and vasomotor collapse ensue; and death occurs, as a rule, from the 10th to the 17th day. In our series, 81 per cent of the deaths occurred in this period. The mortality rate ranges from 2 to 10 per cent in the New Guinea region. The rate is high in patients over 40 years of age, and in those whose health prior to the onset of scrub typhus was compromised by debilitating diseases such as malaria or dysentery. The age of the patients in the present series ranged from 18 to 40 years.

Laboratory Data

The Weil-Felix reaction, agglutination of the Kingsbury strain (OXK), tends to become positive about the tenth day and to reach its maximum near the middle of the third week of illness. In the current series, the maximum titer was 1:1280, but titers as high as 1:50,000 have been recorded. A single agglutination of 1:160 is regarded arbi-

trarily as diagnostic although a lower initial titer may be considered significant if there is a subsequent rise. A negative Weil-Felix reaction does not preclude the diagnosis of scrub typhus inasmuch as rickettsiae have been recovered in such cases by the inoculation of blood into white mice. There is no parallel between the height of the titer of the Weil-Felix reaction and the severity of the disease. Anemia and leukopenia are usually present, particularly early in the disease. The differential count is inclined to be normal but may show a relative lymphocytosis. In severe cases the urine usually contains albumin (2 plus to 4 plus); granular casts are common. The data available to us on the chemical state of the blood and on the cerebrospinal fluid are insufficient to serve as a basis for conclusions.

Observations

Skin

Eschar

The lesion produced by the bite of the mite infected with rickettsiae produces in rapid order a papule, vesico-papule, excoriated papule, frank eschar, ulcer, and finally a small scar. This evolution of the lesion takes place in about 3 to 4 weeks, but may be prolonged if modified by secondary infection such as is prone to occur in the groin or axilla. The maximum diameter of the eschar averages approximately 5 mm. and is surrounded by an erythematous areola of about the same diameter. An eschar was recorded as having been present in 94 per cent of the cases. In 4 of our cases, the eschar was removed during life without any noticeable effect on the course or pathologic features of the disease. In 2 cases, multiple (2) eschars were observed.

Eleven eschars from this series were available for histologic study. In addition, 9 eschars from other cases of scrub typhus were examined. Sections of other stages of the lesion were not secured.

The distribution of the eschars was as follows:

Location	*Cases*
Genitals	11
Axilla	11
Thigh	9
Abdomen	8
Knee and popliteal space	7
Chest	5
Groin	5
Miscellaneous	16
(Back, 3; neck, 3; ankle, 3; buttock, 3; arm, 2; lower leg, 1; heel, 1)	

Histology of Eschar

The eschar appeared to begin as a small intra-epidermal pressure vesicle. The vesicle then became purulent and advanced aggressively in all directions. The contents included serum, intact as well as karyorrhectic polymorphonuclear leukocytes mixed with various mononuclear cells, fragments of keratin, blood, and colonies of bacteria, principally staphylococci (Fig. 1). At this stage, the histologic picture simulated that of hydroa aestivale, a crusting vesicular eruption related to exposure to sunlight. The roof of the vesicle, which was constantly parakeratotic in contrast to the nonnucleated stratum corneum of the nearby epidermis, was disrupted in all of the specimens. In extending inward toward the corium, the pustule tended to destroy the base originally formed by the spinous layer of epidermis, so that after a week or so the dermis formed the floor of the ulcer and the lesion could no longer be recognizable as primarily intra-epidermal. However, even in such advanced lesions, a clue to the morphogenesis of the lesion was found occasionally at the angles of the eschar, where the vesicle might still be seen intra-epidermally, usually with floating wisps of keratin as the remnants of the roof (Figs. 3 and 4).

The epidermis adjacent to the ulcer was usually slightly to moderately acanthotic, with intracellular edema of the rete malpighii and a tendency toward hyperchromatism, some loss of polarity, and crowding of the cells of the basal layer (Figs. 4 and 5). The edema of the papillary layer adjacent to the necrotic tissue was usually slight but in one instance was so marked as to have caused vesiculation.

The suppuration and necrosis extended through the papillary layer to about the mid-corium, as a rule (Fig. 1). In one of the cases in which the skin was from a site normally very thin, the necrosis reached the subcutaneous fat. In practically all instances the suppurative portion of the lesion, while not sharply demarcated, was distinctly zonal. Beyond this zone the infiltrate tended to be localized to the appendages and vessels, and was composed almost exclusively of various mononuclear cells (Fig. 6). The collagenous fibers at the base of the lesion showed a form of degeneration that is seen following severe, acute injury, such as may be produced by chemical, thermal, physical, or electrical agents. The fibers were swollen, acidophilic, homogeneous, and showed loss or distortion of nuclei. We have observed similar coagulative changes following other bites, for example, spider bites. There were also small hemorrhages within and immediately below the floor of degenerated collagen.

The dense collections of mononuclear cells about the coils of sweat glands, hair follicles, sebaceous glands, nerves, arteries, and veins were in striking contrast to the polymorphonuclear reaction in the upper

zone. These mononuclear cells included lymphocytes, plasma cells, many mast cells, and various forms of large macrophages (Fig. 6). Some of these histiocytes were binucleated so as to resemble the Sternberg-Reed cell, a type of cell, incidentally, which we have observed in the cutaneous reactions to tick bites. Unlike the latter reaction, eosinophilic leukocytes were practically absent in the eschar of scrub typhus (Fig. 7). In one case there was a focus of degeneration of dermal collagen containing a fragment of the mite and surrounded radially by foreign body giant cells (Fig. 8). The vessels throughout the corium, beneath and adjacent to the main site of the lesion, were dilated and hyperemic. Within the upper acute inflammatory zone, there was evidence of acute thrombophlebitis and arteritis such as would be seen in any purulent focus. However, of considerable interest were the veins which were located at a distance from the zone of suppuration and necrosis, but which, nevertheless, showed evidence of intimal damage. There were small collections of mononuclear cells—lymphocytes, plasma cells, and macrophages—which infiltrated the intima and lifted up the endothelium over them (Figs. 9 and 10). A single cross section of vein might have two or three such cellular, intimal mounds. Subendothelial vacuolization, such as may be found in association with allergic reactions, as in an asthmatic lung, was observed. In some instances, endothelial cells of veins were enlarged and hyperchromatic. Occasionally a small, nonocclusive thrombus was attached, but frequently there was a focal disintegration of the intima causing a little hillock to form (Fig. 10). This intimal cushion simulated a thrombus but appeared actually to represent an intimal verrucal swelling such as occurs in cardiac valves.[5] This type of phlebitis was found in other organs quite apart from suppurative foci and may presumptively be attributed to the direct action of rickettsiae rather than to the secondary contiguous effects of the purulent reaction of the eschar. Giemsa stains revealed bodies which we interpreted as rickettsiae in venous endothelium in only one eschar. In addition to the intimal verrucae, platelet thrombi also were seen.

Macule of Scrub Typhus

Sections of the rash were available from 9 cases of scrub typhus. These sections were secured at autopsy of patients of whom 7 were ill from 11 to 14 days; of the 2 remaining, 1 was ill 2 days, and the other survived 26 days. With the exception of the patient whose illness lasted 2 days, the histologic picture was essentially similar in all cases. The epidermis was not remarkable. There was moderate edema of the papillary layer with marked hyperemia of the upper layer of the corium. The arterioles, capillaries, and veins were surrounded, gener-

ally eccentrically, by mononuclear cells consisting of lymphocytes, plasma cells, numerous mast cells, and various macrophages. These collections constituted the equivalent of the "typhus nodule" (Figs. 11 to 13). In the one instance of the patient who died after 2 days, there were included in the infiltrate about some of the vessels considerable numbers of polymorphonuclear leukocytes.

The vascular changes were confined to capillaries, venules, and veins. Small platelet thrombi were observed in the vessels in each case. Other vascular changes consisted of enlargement and hyperchromatism of one or more endothelial cells of a capillary or vein, occasionally associated with subendothelial vacuolization or edema, necrosis and swelling of the underlying intima. These changes might or might not be associated with an infiltration of several lymphocytes, plasma cells, or macrophages, and verrucal outpouching of that portion of the intima. Frequently several such hillocks were seen in the same cross section of a vein. As a rule, such phlebitic areas were focal, the intervening portions of the wall of the vein usually appearing quite normal, even within the same plane of the section. Moreover, there was no constant relationship between the reaction in the vessels and the degree of perivascular infiltration or other extra-vascular, contiguous areas of inflammation; rather, these changes appeared to constitute an expression of intrinsic vascular, usually intimal damage. Veins of the hypodermis, fascia, and underlying skeletal muscle were similarly involved.

The appendages were not remarkable except for the frequent degenerative changes in the coils of the sweat glands. These changes ranged from epithelial vacuolization to pyknosis of the nuclei and desquamation of the fragments of the lining cells (Fig. 15). However, these findings were nonspecific and may be observed in many dermatoses. Frequently there were small mononuclear foci of panniculitis, occasionally associated with atrophy of the included fat.

Macule of Louse-Borne Typhus

The macule of epidemic typhus was histologically essentially similar to that of scrub typhus, although several differences were found. In the first place, although capillary thrombi were present in all of the available sections of macules of scrub typhus as against only 15, or 65 per cent, of the macules in epidemic typhus, the thrombi were considerably more conspicuous in the latter disease. They were more prominent not only because more thrombi occurred in a single section, but because they were larger and were associated with more pronounced endothelial changes. In epidemic typhus, the affected endothelial cells tended to be larger, more hyperchromatic, and more often disintegrated into chromatin dust, the last being a feature observed also in the capillaries of

other organs, including the glomerular capillaries. Secondly, there was a tendency to the occurrence in epidemic typhus of a necrotizing arteritis and thrombo-arteritis, not found in scrub typhus. The necrosis might extend through the entire wall of the artery, unlike the lesser degree of involvement that is stated to occur in the experimental animal infected with the rickettsiae of epidemic typhus.[6] Indeed, in the human skin of cases of epidemic typhus there may be infarct-like hemorrhagic suppurative necrosis of the portions of corium in association with severe arteritis. Thirdly, whereas no significant changes were noted in the epidermis of the macule of scrub typhus, minor changes consisting of focal spongiosis, patchy parakeratosis and focal "liquefaction degeneration" of the basal layers were infrequently observed in the skin of patients with louse-borne typhus.

Macule of Rocky Mountain Spotted Fever

The changes in the skin of patients with Rocky Mountain spotted fever approached more closely those of epidemic typhus than those of scrub typhus. However, the scrotal changes were generally considerably more advanced. There was a marked tendency for scrotal hemorrhage and actual gangrene to occur. The basis for the necrosis was found in extensive, necrotic panarteritis and thrombo-arteritis of the corium. Frequently the necrotic vessels were surrounded by an exudate in which the polymorphonuclear leukocyte predominated. These gangrenous areas were superficially reminiscent, from a histologic point of view, of the primary eschar of scrub typhus, but there were several important differences, especially the absence of thrombo-arteritis at a distance from the gangrenous area of the eschar. In less markedly involved arteries in spotted fever, the walls were focally infiltrated with mononuclear cells and polymorphonuclear leukocytes, often associated with fibrinoid necrosis of part of the wall and much karyorrhexis of the indigenous as well as the infiltrating cells of the vessels.

In addition to the macules of Rocky Mountain spotted fever, there were available sections of macules from 17 cases of fièvre boutonneuse. As might be expected, these were quite similar to those of Rocky Mountain spotted fever except, perhaps, for the greater edema of the papillary layer and the more frequent foci of acute panarteritis in fièvre boutonneuse. No sections of the primary lesion (tache noire) were studied.

HEART

Myocardium

Sections from 74 hearts were examined and in most instances multiple sections were available. Of these, only 5, or 7 per cent, were con-

sidered essentially negative. In the remainder, various degrees of interstitial myocarditis were present. The cells of the infiltrate consisted of small and large lymphocytes, plasma cells, several kinds of mononuclear phagocytes, perhaps in different stages of development, Anitschkow myocytes, scattered mast cells, and infrequently neutrophilic and eosinophilic leukocytes. The character of the infiltrate resembled that in other organs, except that the myocyte was confined to the heart. "Giant cells" were formed by binucleate plasma cells or macrophages. Occasionally the macrophages showed evidence of erythrophagocytosis and cytophagocytosis. The type of infiltrate was similar in the three varieties of typhus, although polymorphonuclear leukocytes were prone to occur more often in spotted fever. The infiltrate was located principally between muscle fibers, although it was

TABLE II

Comparative Degree of Interstitial Myocarditis

Disease	0		+		++		+++		++++		Total cases
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	
Scrub typhus	5	7	32	44	27	35	8	11	2	3	74
Epidemic typhus	4	17	12	50	5	21	3	12	0	0	24
R. M. s. f.	2	17	7	58	3	25	0	0	0	0	12

frequently found also in the periarterial fibrous tissue and rarely within the sarcoplasm of a muscle fiber (Fig. 16). Usually the infiltrate was associated with an interstitial edema recognizable by the loose disposition of the fibers and the presence of fine granular protein precipitate in the interstitium. The infiltrate varied in quantity from about one focus of several cells in each low-power field to a striking concentration in which the cells occupied approximately a third of the field. The degree of infiltration was graded 1 plus to 4 plus on the basis of these approximate limits and compared with the corresponding changes in the hearts from cases of epidemic typhus and Rocky Mountain spotted fever. The criteria for the different grades of infiltration are obviously not precise but, nevertheless, Table II does provide an index of the relative degree of myocarditis in these three conditions.

Although 12 cases of the tick-borne disease constitute a small series, it appears from this table that the myocardium in both scrub and epidemic typhus tends to suffer greater involvement than it does in spotted fever. Similarly, 49 per cent of the cases of scrub typhus have 2 plus or greater degrees of infiltration, whereas only 33 per cent of the epidemic type are in the same category. It seems, therefore, that the degree of myocarditis, in descending order of involvement, is: (1) scrub typhus; (2) epidemic typhus; (3) spotted fever.

Localization of Myocarditis. In five instances, sections from both ventricles were available. There was no evidence to support the impression that the right heart was involved more frequently than the left, that the ventricles were more severely damaged than the auricles, or that one part of the wall of the ventricle was selectively involved. However, it is true that in a given case there usually was an unequal distribution of infiltrate. This obtained not only in sections taken from different locations, but even within a single section the infiltrate might be collected in one part of the section and spare a considerable portion of the remainder. Because of this uneven distribution of myocarditis, an erroneous impression of a selective localization of infiltrate might be acquired from the examination of an insufficient number of slides. Moreover, just as the quantity of infiltrate varies, so may its character. That is to say, in one section, the plasma cell may be the predominant element; in another, the acidophilic macrophage may be most numerous; and in still another section from the same case, the Anitschkow myocyte may predominate.

Integrity of Myocardial Fibers. Notwithstanding the abundance of infiltrate, it is our impression that, with one qualification, there is a remarkable preservation of myocardial fibers in all three types of typhus. Exceptionally, there was found an isolated, swollen, partially hyalinized fiber, in the sarcoplasm of which there were one or two karyorrhectic inflammatory cells; or, rarely, a group of fibers interspersed with mononuclear cells showed evidence suggestive of an ischemic atrophy (Fig. 20). However, the impression of the morphologic integrity of the fibers must be qualified by the occurrence of fragmentation of the fibers, particularly at the sites of inflammatory foci (Fig. 16). Although the markings of such fibers remained distinct, the fragmented edges frayed off into fine fibrils which were then lost in the inflammatory zone. Small hemorrhages were rarely observed in these areas or, indeed, elsewhere in these hearts. It is acknowledged, of course, that the use of the diagnosis "fragmentation of myocardial fibers" is much overdone because the change is generally post-mortem or at most agonal. Nevertheless, we should be hesitant to dismiss summarily this particular form of fragmentation because of its association with foci of infiltrating cells.

Myocardial Vessels. Although the character of the infiltrate and the changes in the myocardial fibers are essentially similar in scrub typhus, epidemic typhus, and spotted fever, there are distinct differences in the involvement of the cardiac blood vessels. In the hearts of scrub typhus no artery was found showing the obvious fibrinoid degeneration that may be seen occasionally in the other types of typhus. In only one instance was an intramyocardial artery found infiltrated with mononuclear cells, localized almost exclusively to the intima. Similar subendo-

thelial foci were found in several of the main coronary arteries and in the aorta. This form of infiltration can be found in other infectious diseases. A small nonocclusive thrombus was present in one of the coronary arteries. Occasionally the endothelium of capillaries, arterioles, and arteries might be swollen and lifted off the intima by subendothelial edema. In two instances there was observed focal thrombophlebitis of small myocardial veins. Occasionally the arterioles manifested a focal, verrucal, acellular, intimal swelling which, again, was of the type seen in a variety of infectious states. Such completely nonspecific intimal swellings are associated with thrombi or so-called "granular plugs," [5] thus complicating the use of one of the principal diagnostic features of the typhus diseases.

Whereas no instance of necrotizing arteritis was found in the myocardium of scrub typhus, in 4, or 17 per cent, of the cases of epidemic typhus such examples were found. The arteritis involved the small vessels and varied in degree from focal inflammation of the intima to a marked necrosis extending not only through the entire wall but to the surrounding periarterial collagenous mantle (Fig. 22). The walls of such vessels were infiltrated with various mononuclear cells, some of which were markedly distorted or actually karyorrhectic. In places, the necrosis was of such severity as to have destroyed even traces of the normal cells of the vascular wall. This type of lesion closely resembled the arteritis seen in some of the diffuse vascular diseases such as disseminated lupus erythematosus. Focal mononuclear phlebitis, associated usually with a nonoccluding platelet thrombus, occurred about as often as arteritis in our cases of epidemic typhus. In the cases of spotted fever, vascular lesions of the heart, exclusive of the slight to moderate swelling of capillary and arteriolar endothelium, were rare and consisted of a focal, mild, mononuclear infiltration of veins with thrombosis, very much of the order of change observed in scrub typhus.

Finally, it was noted that the intramyocardial nerves may participate in the inflammatory reaction in each of the three diseases (Fig. 21). Similar neuritis was found in other organs.

Endocardium

The involvement of the mural endocardium by the mononuclear infiltrate was often striking in scrub typhus and was observed in about one-third of the cases. The infiltrate tended to be more marked in the endocardium over the papillary muscles or columnae carneae. In its severest form, the mononuclear infiltrate of lymphocytes, plasma cells, and macrophages occupied the entire thickness of the endocardium. Occasionally the infiltrating cells were palisaded so as to simulate a rheumatic reaction. In its milder forms, it was not unlike the reaction

seen in other infectious diseases or following the use of serum.[7] In only one instance was a mural thrombus observed, although the presence of small pulmonary emboli in several other cases suggested the occurrence of mural thrombi in additional instances. In one case a small verruca of the mural endocardium was formed at the site of degeneration of the endothelium. As a rule, the endothelium remained intact over the infiltrate. No correlation generally was found between the degree of infiltration of the myocardium and the endocardium. The valves showed a similar infiltrate in the auricularis layer of the mitral (4 of 9 cases) and in the ventricularis of the aortic valve (3 of 4 cases). In one instance, the cellular infiltration of the spongiosa of the mitral valve was so extensive as to be of itself indistinguishable from rheumatic valvulitis. In no instance were Aschoff bodies found although the edema of the interstitium and proliferation of histiocytes and Anitschkow myocytes might superficially simulate an Aschoff body. In 2 cases, the interstitial mononuclear reaction was associated with fibrinoid degeneration of the collagen so as to suggest the interstitial myocarditis of disseminated lupus erythematosus even more strongly than that of rheumatic fever.

The endocardial infiltrate in the cases of *epidemic typhus* was of similar quality but far less intense. The endocardium showed no significant reaction in our series of cases of *spotted fever*.

Epicardium

In the epicardium were found focal perivascular infiltrations of mononuclear cells of the same type as in the myocardium. Frequently, there was a preponderance of eosinophilic macrophages, as in other infectious diseases and, incidentally, as in cases of lupus erythematosus. These cells infiltrated fat about other organs as well, *e.g.*, the perirenal fat. In several instances there was focal serous atrophy of the fat. Mononuclear cell infiltration of an epicardial nerve occurred occasionally. In only a single case was intrinsic vascular involvement seen and then merely in the form of a focal thrombophlebitis. The epicardial lesions of *epidemic typhus* and *Rocky Mountain spotted fever* are basically similar to those of scrub typhus. In one case of epidemic typhus there was observed a striking fibrinoid degeneration of a small coronary artery and adjacent fat (Fig. 25).

AORTA

The aorta frequently showed small subendothelial collections of lymphocytes, plasma cells, and macrophages. Similar cells were located about the vasa vasorum in the adventitia and outer media, often

with slight fragmentation of the elastic fibers in the vicinity. These lesions only remotely resembled the mesaortitis of syphilis. Similar lesions were seen in the aorta in cases of *epidemic typhus* and *spotted fever*.

THE LUNGS IN SCRUB TYPHUS

Interstitial pneumonitis of various degrees of intensity was found in 55 per cent of cases of scrub typhus. The earliest stage of the pneumonic reaction appeared to be a marked dilatation and hyperemia of septal capillaries followed by extensive extravasations of masses of red blood cells into the alveoli, often with an admixture of serum and a few mononuclear cells. The septa in this phase, although hyperemic, were not significantly infiltrated with inflammatory cells. The large mononuclear macrophages increased in number and lay scattered or clumped within the alveoli. These cells often contained fat and hemosiderin and might closely simulate "heart lesion" cells, especially in association with the hemorrhagic extravasations. In other areas, the alveoli might be distended merely with serous fluid. Even in these early phases there were detectable single, large, mononuclear cells with basophilic cytoplasm and hyperchromatic swollen nuclei, usually adherent to the septal wall or emerging from septal capillaries. The bronchiolar walls might be infiltrated almost exclusively with lymphocytes, plasma cells, and macrophages whereas the lumen might be filled with purulent exudate. The fasciculi of muscle of the bronchioles were often disrupted by much edema and infiltrate.

In a later stage, the septa became conspicuously thickened (two to four times) by the mononuclear cells as well as by scattered polymorphonuclear leukocytes. In small foci of atelectasis, collapsed, apposed septa might simulate actual reactive thickening of the septa. Septal capillaries were discernible but generally not hyperemic. An alveolar lining of mononuclear cells might or might not contain the mononuclear phagocytes with varying amounts of edema fluid. The alveolar fluid showed a tendency to inspissate in crescentic form adjacent to the septal wall. The interlobar septa were usually markedly edematous (Fig. 32). Purulent or nonpurulent exudate was frequently present in the bronchioles, and polymorphonuclear leukocytes might be scattered through the mononuclear infiltrate of the bronchiolar wall. In addition, there might be several alveoli or actual lobules filled with purulent exudate and representing a secondary, bacterial bronchopneumonia adjacent to a focus of interstitial pneumonitis. Commonly, areas of emphysema accompanied the pneumonitis.

At the height of the reaction, the alveoli were rimmed prominently by the mononuclear macrophages (alveolar "epithelium"). Some of

LIVER

Scrub Typhus. Sections of liver from 70 cases were examined. The changes of principal interest were as follows:

1. An almost constant erythrophagocytosis and cytophagocytosis by swollen Kupffer cells.

2. An excess amount of fat, principally periportal, in 13, or 19 per cent, of the cases.

3. An increase in the sinusoidal cells. These cells include the swollen Kupffer cells, lymphocytes, plasma cells, and basophilic macrophages.

4. Foci of necrosis in a small number of cases (6 cases or 9 per cent) (Figs. 37 to 39). These foci averaged about the diameter of a pancreatic islet although in one case they were several times that size. They consisted of partially or completely lysed parenchymal cells with collapsed or dilated sinusoids. There often seemed to be a focal increase of Kupffer cells, but this impression was due merely to an apparent, rather than actual, increase of these cells following the collapse of the sinusoids and the loss of hepatic cells. In some foci there was an accompanying infiltration of lymphocytes, plasma cells, and macrophages, and occasionally polymorphonuclear leukocytes. Of interest were the small clumps of vesicular cells that seemed to be forming abortive biliary canaliculi within these foci. A few of these lesions were necrotic and composed of circumscribed eosinophilic, granular, and fibrinoid material. In one instance, the lesion resembled a typhoid nodule. In addition to the focal lesions, there was marked central congestion of sinusoids with associated necrosis of parenchymal cells in three instances. In one case there was a concomitant, extensive polymorphonuclear exudation about the central veins. This alteration is identical to that observed in shock or severe passive congestion and is attributed to anoxia of the central portion of the lobules. In addition, dissociation of hepatic cords without other alterations was frequently observed. This change of normal pattern, seen in other types of sepsis as well, is attributed to post-mortem alterations.

5. Finally, there was a slight to moderate tendency toward an increased concentration of various mononuclear cells in the periportal areas.

The changes in the livers of *epidemic typhus* and *Rocky Mountain spotted fever* were essentially similar to those of scrub typhus. However, the impression was gained of a more vigorous reaction in those diseases than in scrub typhus. That is to say, the cellularity of the portal areas tended to be greater; the foci of necrosis, while of about the same frequency, were more likely to include polymorphonuclear leukocytes and to a more conspicuous degree; and phlebitis and arteri-

tis, though by no means marked, were more apparent than in scrub typhus.

SPLEEN

Scrub Typhus. The findings in the spleen resembled closely the proliferative form of "acute infectious splenitis" or "acute splenic tumor" such as is seen in typhoid fever, for example. The follicles tended to be small for the age group concerned. Unlike the picture in some other acute infectious diseases (*e.g.*, diphtheria), the follicles were more likely to be intact than to manifest central hyalinization and necrobiotic changes. Both the Billroth cords and the sinuses were inclined to be hyperemic, although frequently they contained many large mononuclear cells. Polymorphonuclear leukocytes were relatively uncommon. The predominant cell in the sinuses and the cords was the basophilic macrophage, which in this organ, particularly, has been called endothelial phagocyte, basophilic histiocyte, and acute splenic tumor cell. This is the cell which Rich,[42] on convincing evidence, has linked with the lymphoblastic series. Erythrophagocytosis and cytophagocytosis were almost constantly present, often to a pronounced degree. Of the 68 cases from which sections were examined, 5, or 7 per cent, showed definite evidence of necrosis of the pulp, ranging from small foci to large, irregular, infarct-like areas, in most respects resembling the necrosis of the lymph nodes. Isolated thrombophlebitis of trabecular veins was found in 4 cases. No association was found between the phlebitis and the splenic necrosis. In the one smear of spleen submitted, stained by the Giemsa method of Wolbach, rickettsiae were found in cells that appeared to be macrophages (Fig. 44).

The sections of spleen of both *epidemic typhus* and *Rocky Mountain spotted fever* were quite similar to those of scrub typhus with the exception of a more manifest tendency toward relatively greater numbers of polymorphonuclear leukocytes in the pulp, especially in epidemic typhus. In one case of epidemic typhus there was marked fibrosis of Billroth cords. No malarial pigment was present. In a case of Rocky Mountain spotted fever in which sickling of red blood cells was evident in the bone marrow (white male), perifollicular collections of blood were noted in the spleen. This finding was absent in the spleens in those cases of scrub and epidemic typhus in which sickling was observed in other organs.

LYMPH NODES

Scrub Typhus. Sections of lymph nodes from 47 cases were available. The changes were those due to hyperplasia or necrosis. In all instances, there were varying degrees of hyperplasia; in 18, or 38 per

cent, of the cases, the hyperplasia was accompanied by necrosis. The hyperplastic nodes were characterized by marked distention of the sinuses, principally with acidophilic macrophages. Lymphocytes and plasma cells, as well as clumps of fibrin and red blood cells, were also often included. The cytoplasm of the acidophilic histiocytes was usually granular, or finely vacuolated ("blister" histiocytes) and usually exhibited striking erythrophagocytosis and cytophagocytosis, both in the pulp and sinuses (sinus catarrh). The necrosis of the nodes ranged from focal karyorrhexis, hemorrhage, and deposits of fibrin and edema within a germinal center or sinus, to a massive, infarct-like involvement of almost an entire lymph node. Necrosis was present not only in nodes regional to the site of the eschar, but also in those in other areas. Frequently in the necrotic nodes the endothelium of capillaries and veins was swollen and karyorrhectic, occasionally with overlying small platelet thrombi. In the more intensely involved nodes, the mononuclear reaction was prone to extend through the capsule into the adjacent areolar tissue.

Unlike those of scrub typhus, the lymph nodes of both *epidemic typhus* and *Rocky Mountain spotted fever* did not show necrosis. They were generally moderately hyperplastic. The sinuses were usually packed with acidophilic macrophages showing abundant evidence of phagocytosis. In one of our cases, the sinuses of a pulmonary lymph node were filled with purulent exudate, apparently reflecting the purulent bronchopneumonia present in this case.

PANCREAS

Scrub Typhus. Sections of pancreas were available in 45 cases. In 14, or 31 per cent, there were foci of interstitial reaction consisting of lymphocytes, plasma cells, and macrophages, and, in 1 case, of polymorphonuclear leukocytes. These foci were never striking and appeared to be merely a part of the general perivascular and interstitial reaction that characterizes this disease. In 1 case, squamous metaplasia of the pancreatic ductules was observed.

The sections of pancreas in *epidemic typhus* and *Rocky Mountain spotted fever* were quite like those of scrub typhus.

ADRENAL

Scrub Typhus. Sections of the adrenal gland were examined in 52 cases. The principal changes were: (1) focal infiltrations of lymphocytes, plasma cells, and macrophages, preponderantly in or about the medulla, but also in the cortex and areolar tissue; and (2) a practically constant finding of varying degrees of so-called "tubular" degenera-

tion,[10] principally of the fascicular cords (Fig. 49). Our clinical data were inadequate for a study of the correlation between the degree of tubular degeneration of the adrenal glands and shock. A third finding of note was the zonal distribution of abundant fat in the mid-fascicular layer in 7, or 13 per cent, of the cases. In the remainder of the cases, the fat content seemed sparse, in keeping with the usual status of the adrenal in infectious diseases. Finally, slight focal thrombophlebitis was present in 3 cases; in 1 case, the main adrenal vein and its tributaries were thrombosed, with resultant infarction of most of the gland.

The adrenal glands in *epidemic typhus* were essentially similar to those of scrub typhus, although the focal mononuclear reaction was generally more conspicuous. The one significant point of difference was the occurrence in epidemic typhus of readily apparent inflammation and degeneration of capillaries and arterioles of both the parenchymal and adventitial tissues in 4 of the 24 cases. The adrenals of the cases of *Rocky Mountain spotted fever* were basically similar to those of scrub typhus. In only 1 of the cases was evidence of thrombo-arteritis seen.

KIDNEY

Scrub Typhus. Glomeruli. Evidence of early, but definite, acute diffuse glomerulonephritis was found in 19, or 30 per cent, of 64 cases examined. In some of these kidneys, the glomeruli were moderately to markedly enlarged (Fig. 52); in the majority, they were of normal size. However, regardless of the size of the glomeruli, there were, commonly, hyperplasia, hyperchromasia, and enlargement of the endothelial cells; similar changes of a lesser degree were observed in the epithelial cells. In addition, the epithelial cells of both the parietal and visceral layers of Bowman's capsule frequently showed acidophilic swelling and granular disintegration of the cytoplasm. In a few instances, the damage to the glomerular capillaries had progressed to the stage of actual karyorrhexis of the endothelial nuclei, with the formation of isolated platelet thrombi. (This degree of damage was more common in epidemic typhus.) In sections stained with hematoxylin and eosin, the impression was gained in many instances of a marked granular or hyaline thickening of the basement membrane of the glomerular capillaries. However, in sections stained with Mallory-Heidenhain's azocarmine and Masson's trichrome stains, it was found that the apparent thickening was produced generally by a syncytium of endothelial cells. However, in a few cases there was slight to moderate, uniform, hyaline, refractile thickening without conspicuous reduplication. The major common denominator of all these cases of glomerulonephritis was the moderate to complete ischemia of the

glomerular capillaries. In some cases, practically every glomerulus in the section was so affected; in others, although most of the glomeruli were ischemic, the capillary status of the remainder ranged from partial ischemia to marked engorgement. In some individual glomeruli, the majority of the loops were significantly devoid of red blood cells, although isolated loops were distended with blood. In several cases, the glomerular loops were fused to each other (Fig. 52); only rarely was a capillary loop found adherent to Bowman's capsule. In no instance was there evidence of the formation of glomerular crescents.

The histologic picture of glomerular ischemia assumed one of two forms: (1) The capillaries were distended with protein precipitate, fibrin, or, infrequently, by platelet thrombi; (2) the lumina of the capillaries were encroached upon, and practically obliterated, by enlarged, hyperchromatic endothelial cells (Fig. 53). Commonly, the afferent arteriole was appreciably dilated, ischemic, and filled with protein precipitate. Bowman's space was often distended, at times markedly, and sometimes contained much protein precipitate, both of the granular variety and in large, agglutinated, acidophilic masses. In several instances Bowman's capsule showed fibrinoid degeneration of the type seen in the acute glomerulonephritis associated with other diseases and in the renal lesions of disseminated lupus erythematosus (Fig. 53).

In 7 cases, changes qualitatively similar to those just described were confined to scattered glomeruli. These kidneys were diagnosed as acute focal glomerulonephritis.

Tubules. In practically all of the cases, there was swelling of the epithelium of the proximal convoluted tubules by cytoplasmic granules; or, in the more advanced changes, by hydropic vacuoles. These vacuoles, unlike those due to fat, appeared first at the luminar rather than basal aspect of the cell, and thence might extend to the entire cell. The vacuolization was often associated with collections of large, acidophilic hyaline droplets, located also near the lumen of the tubule rather than near the bases of the cells. These colloid droplets were morphologically identical with those seen commonly not only in acute glomerulonephritis, but also in lipoid nephrosis and following the administration of hypertonic sucrose.[11] Fat stains revealed only a few small droplets at the bases of the epithelial cells; the vacuoles alluded to above did not contain fat. No correlation was found between the degree of vacuolization and the presence of glomerulonephritis. The lumen of the proximal portion of the nephron was filled almost constantly with protein precipitate. Occasionally the proximal loops were appreciably dilated.

The principal change observed in the distal segment of the nephron was the presence of casts of hemoglobin in 44 cases, or 69 per cent. One or two such casts were usually found in the distal loops, appearing as satellites to the glomeruli, so to speak. In most cases, several nephrons in a section were so involved and were often associated with iron pigment in the tubular epithelium.[12] In a few cases only an occasional nephron failed to show such a cast. No correlation was found between the presence of these casts and glomerulonephritis or interstitial nephritis. In no case was there any indication that hemoglobin casts, *in this particular location,* were responsible for tubular blockage, epithelial damage, or renal insufficiency. However, in 2 cases, there was an obvious hemoglobinuric nephrosis characterized by numerous casts of hemoglobin in the *collecting tubules* as well as in the distal convoluted tubules, degeneration and regeneration of this portion of the nephron, dense acidophilic casts adjacent to necrotic distal tubules, phlebitis, and focal interstitial nephritis. In neither of these cases was there a record of the administration of a sulfonamide drug or of transfusions. On the other hand, in both of the cases there was an acute diffuse glomerulonephritis; and, indeed, in one of them, a conspicuous degeneration of the epithelium of the proximal convoluted tubules. In 3 other cases of acute diffuse glomerulonephritis, there was appreciable degeneration of the epithelium of the distal tubules *without* an associated hemoglobinuric nephrosis. Finally, in 4 cases, several small, amorphous, deeply basophilic, as well as crystalline spherules, each about the size of a monocyte, were found in the lumen of the distal convoluted tubules. These bodies were suggestive of crystals of calcium oxalate. In none of these 4 cases was there a history of sulfonamide administration; in 2, acute diffuse glomerulonephritis was present; in the other 2 cases there was merely focal interstitial nephritis.

Interstitium. Foci of interstitial nephritis of varying degrees constituted the most constant alteration of the kidney of scrub typhus. The infiltrate was most prone to occur at the cortico-medullary junction but might occur anywhere from the pelvis to the capsule. In the kidney, as in the other organs, the infiltrate was almost entirely mononuclear, consisting of lymphocytes, plasma cells, and basophilic histiocytes; occasionally a few neutrophilic and eosinophilic leukocytes were included. The infiltrate appeared to select particularly the walls of the veins and the perivenous tissue, as in other organs. In the cortex, especially, the interstitial infiltrate was often associated with considerable edema (Fig. 60). In 1 case, the infiltrate and edema were uniform and diffuse so that the diagnosis of acute interstitial nephritis was

warranted, although the association with a concomitant acute diffuse glomerulonephritis bespeaks a pathogenetic relationship.

Vessels. If the testis is excepted, vascular changes in scrub typhus were most common in the kidney. The changes assumed one of the following forms:

1. Swelling, hyperchromasia, hyperplasia, and rarely karyorrhexis of the endothelial cells of glomerular capillaries.

2. Thrombophlebitis, especially of the venulae rectae and interlobar veins, usually in association with adjacent interstitial mononuclear infiltrate which had extended into the wall and puffed out the intima into a focal hillock (Fig. 61). Other veins showed a local intimal vacuolization, fibrinoid degeneration, and infiltration of a few lymphocytes, plasma cells, or macrophages, unattended by periphlebitis. The endothelium might be either intact or degenerated; in the latter case, a small platelet thrombus might be present.

3. Arteriolonecrosis in 3 instances. Segmental necrosis of arterioles was observed in the midst of foci of mononuclear, interstitial infiltrations. In none of these cases was there glomerulonephritis or hypertension. In 1 of the cases, sulfathiazole and quinine had been administered; in the other 2, no history of chemotherapy was recorded.

In addition to the above alterations, there were two findings of note in the contents of the renal veins: (1) In 8 cases, all white soldiers, distinct evidence of sickling of red blood cells was found in the lumen of one or more of the interlobular veins and rarely in the arcuate arteries. There seemed to be no definite relation between sickling and the presence of glomerular lesions. In 4 of these cases, sickling was absent in other organs: in 2, sickling was found in the lungs; in the third, a lymph node; and in the fourth, a testis. No perifollicular lakes of blood, such as characterize sicklemia, were found in these cases. (2) The second feature of interest in the contents of the vessels was the marked concentration of mononuclear cells in the peritubular venules of the medulla. Frequently the lumina were filled with lymphocytes, plasma cells, and basophilic and acidophilic macrophages, the latter, especially, showing phagocytosis of red and white blood cells (Fig. 57). In many of these vessels polymorphonuclear leukocytes were entirely absent, although present in more or less normal numbers in the peripheral blood. The cells were not agglutinated and were not part of a stratum of a post-mortem clot. These cells corresponded exactly to those observed in the interstitial infiltrate of the kidney and other organs. However, no correlation was found between the degree of adjacent interstitial infiltrate and the concentration of these mononuclear cells in the lumina of the veins. A similar, although generally less conspicuous finding, was noted in other organs.

Epidemic Typhus. The changes in the kidneys from patients with epidemic typhus were qualitatively similar to those of scrub typhus, but considerably more pronounced. Acute diffuse glomerulonephritis was found in 18, or 78 per cent, of the cases; acute focal glomerulitis in 3, or 13 per cent, and essentially normal glomeruli in 2, or 9 per cent. The glomerular alterations were basically those of scrub typhus, differing in more marked swelling and hyperchromasia of the endothelial cells, more frequent occurrence of thrombosis of the glomerular capillaries and fragmentation of the endothelial cells. Similarly, focal interstitial infiltrations, calcific bodies, hemoglobin casts of the distal convolutions, and phlebitis, arteritis, and arteriolitis were more conspicuous in epidemic typhus. Sickling of red blood cells within renal veins was found in 8, or over one-third, of the 23 patients, all of whom were native Egyptians. In 7 of the 8 cases, acute diffuse glomerulonephritis was present; in the eighth, a focal glomerulitis was noted. Sickling was observed in various other organs in these cases although no perifollicular pooling of blood was found in the spleen. Concentration of mononuclear cells, some showing evidence of phagocytosis, was observed with approximately the same frequency and under the same conditions as in scrub typhus.

Rocky Mountain Spotted Fever. The lesions of the kidneys of patients with Rocky Mountain spotted fever were in some respects intermediate between those of scrub typhus and of epidemic typhus. Acute diffuse glomerulonephritis was found in 5, or 50 per cent, of the 10 cases; acute focal glomerulitis in 2 cases, and normal glomeruli in 3 cases. Focal interstitial nephritis, isolated hemoglobin casts in the distal convoluted tubules, concentration of mononuclear cells in the peritubular capillaries, and phlebitis were of the order seen in scrub typhus. A feature of decided divergence was the extensiveness of the necrotizing arteritis observed in the kidneys of patients with spotted fever. Acute necrotizing thrombophlebitis and thrombo-arteritis of the interlobular and arcuate vessels were noted in 3 cases; in 1 case, an infarct of the renal cortex was produced. Sickling was not found in these kidneys, although this phenomenon was manifest in a section of bone marrow from a white patient.

TESTIS

In the histologic study of the effects of generalized rickettsial infection in animals, the testis and its coverings have yielded more information than any other organ because of the selective tendency of certain strains to produce orchitis. For this reason, the morphologic changes in the human testis are of particular interest. In scrub typhus, the changes, as observed in sections of 36 cases, were found to be of no

great differential significance. They consisted of: (1) almost constant, degenerative changes—often present to a marked degree—of the epithelium of the testicular tubules (Fig. 63); (2) edema, early fibrosis, and often marked infiltration of lymphocytes, plasma cells, and various macrophages within the interstitium; (3) occasional hyperplasia of testicular interstitial cells; (4) frequent thrombophlebitis and rare arteritis—both associated with fibrinoid degeneration, edema, and mononuclear cellular infiltration of the wall (Figs. 62 and 79). Arteritis or thrombo-arteritis was present in 5, or 17 per cent, of the cases. Even though slight, this incidence is higher than that observed in other organs. Thrombophlebitis was noted in 7, or 19 per cent. The tunica vaginalis showed simply slight perivascular cuffing by lymphocytes, plasma cells, and macrophages. In the 6 cases from which sections of epididymis were available, slight thrombo-arteritis and thrombophlebitis were noted once. No hemorrhages were observed in the testis or epididymis.

Tubular atrophy, and interstitial edema and infiltration in the testes of patients with epidemic typhus are of about the same order as seen with scrub typhus. However, the point of divergence is the greater frequency and extent of the arteritis in the former. Of the 9 cases from which sections were available, arteritis or arteriolitis was observed in 4, or 44 per cent, a significant difference notwithstanding the small size of the sample. In 3 of these 4 cases, epididymis was included and all showed a similar acute arteritis. In a tenth case, epididymis alone was submitted and here, again, necrotizing arteritis was present. Among the sections of testis in which no arteritis and only a minimal degree of interstitial inflammation were noted, was one from a 10-year-old child. This observation is mentioned because of the tendency for severe involvement of the testes of children with Rocky Mountain spotted fever.[13] The 3 testes of spotted fever which were examined in our series resembled more closely those of scrub typhus than of epidemic typhus. All were from adults, however. The sections of scrotum, on the other hand, showed the characteristically marked changes of Rocky Mountain spotted fever, including hemorrhagic necrosis and acute necrotizing thrombo-arteritis, as described under "Skin."

BRAIN

Scrub Typhus. The involvement of the brain in scrub typhus takes the following forms: (1) mononuclear cell meningitis; (2) "typhus nodules"; (3) perivascular cuffing of arteries; (4) focal hemorrhages in parenchyma and meninges; and (5) degeneration of ganglion cells.

Leptomeningitis was usually present, having been found in 48, or

89 per cent, of the cases (Fig. 73). In 7 cases, the reaction was especially marked. In the 6 cases without meningitis, death occurred on the 2nd, 11th, 13th, and 14th days. In 24, or 44 per cent, meningitis occurred without parenchymal involvement. The degree of meningitis closely paralleled the intensity of periarterial reaction within the brain, but no correlation was found between the meningitis and other parenchymal lesions of the brain. The meningeal infiltrate consisted predominantly of acidophilic macrophages, with a sprinkling of lymphocytes and plasma cells.

The reaction in the parenchyma was characterized by the "typhus nodule" which was present in 18, or one-third, of the cases. The nodule was composed predominantly of oligodendroglial cells, particularly in its earliest or smallest form. In the larger nodules, a few microglial cells, lymphocytes, plasma cells, and macrophages were added (Fig. 67). Rarely, polymorphonuclear leukocytes were included. The number of cells composing the nodules averaged 15 to 20 (in a single plane of section), although the larger nodules contained as many as 40 cells. In 12 of the 18 cases, from 1 to 3 nodules were seen in a section of ordinary size; in the remaining one-third, from 6 to 10 nodules were found in a section. These lesions, as measured in serial sections, approximated 60 to 120 μ in diameter. The glial fibers within and adjacent to the nodules were often edematous or showed early demyelinization. All cases with nodules gave evidence of damage to ganglion cells in the form of satellitosis, particularly within the cortex but also in the pons and basal ganglia.

The cells of the nodule were grouped around capillaries which, however, usually could not be discerned in a single section, especially if stained only with hematoxylin and eosin. Because of the affinity of the basement membrane of capillaries for silver, serial sections of brain were so stained (Wilder stain). As a result, capillaries that were not evident in sections stained with hematoxylin and eosin were unmasked (Figs. 69 and 70). Such capillaries were found constantly, placed either centrally or eccentrically, within the nodule. Frequently the walls of the capillaries were disrupted (Fig. 69). When capillaries were seen in sections stained with hematoxylin and eosin, their walls were often found to be swollen and granular. Endothelial cells, when present, tended to be enlarged and hyperchromatic, as in other organs. The lumina were frequently empty; sometimes a single or several intact or laked blood cells were present; or the lumina were occupied by one or more mononuclear cells, possibly endothelial or hematogenous. Platelet thrombi and karyorrhexis of endothelial cells were not seen. Small, focal, parenchymal, apparently terminal, hemorrhages were ob-

served in 7, or 13 per cent, of the 54 cases. There was no indication of an association between the hemorrhages and the nodules.

The distribution of the nodules was not uniform. The cortex was involved with about one-third the frequency of the pons and medulla. Moreover, the cortical involvement included only the gray matter, the white matter having been spared entirely.

Epidemic Typhus. A comparison between the reactions in the brain and meninges in epidemic and scrub typhus is shown in Table IV. From this comparison, it appears that:

1. Although the meningitis of scrub typhus is slightly more frequent

TABLE IV

Comparative Distribution of Lesions of the Brain in Scrub Typhus and in Epidemic Typhus

	Scrub typhus			Epidemic typhus		
	Total cases	Positive	%	Total cases	Positive	%
Meningeal infiltrate	54	48	89	23	17	73
Nodules						
Cortex, gray	50	5	10	24	20	83
Cortex, white		0	0		0	0
Pons	48	16	33	21	17	81
Medulla	30	10	33	18	14	78
Basal ganglia	24	4	17	15	13	87
Cerebellum	30	3	10	14	11	79
Cord	10	2	20	15	13	87

and extensive than the qualitatively similar reaction in epidemic typhus, the involvement of the substance of the brain is considerably greater in the latter disease.

2. The distribution of lesions in the gray and white matter in the two diseases is the same: in both the white matter is spared, in contrast with Rocky Mountain spotted fever.

3. In the current series the case incidence of involvement of the cortex in epidemic typhus is much greater than in scrub typhus. The actual concentration of nodules in the pons, medulla, and basal ganglia in epidemic typhus is more pronounced than in the cortex. The pons and medulla are sites of predilection also in scrub typhus.

Several distinct histologic differences between scrub typhus and epidemic typhus are noted:

4. The nodules tend to be larger in epidemic typhus, averaging 55 to 75 cells as against 15 to 40 cells, and about 120 to 180 μ as against 60 to 120 μ.

5. There is somewhat greater cellular pleomorphism in the nodules of epidemic typhus, especially in the larger. Karyorrhexis is common

in the cells of the nodule of epidemic typhus and rare in that of scrub typhus.

6. In epidemic typhus, the capillaries of the nodules show much more obvious evidence of damage in the form of markedly enlarged endothelial cells, karyorrhexis of endothelial cells, and thrombosis of capillaries. Similar changes are found in arterioles without associated nodules.

Rocky Mountain Spotted Fever. The sections of brains of only 7 cases were studied. This number is insufficient to permit a tabulation of lesions as was done with the cases of scrub and epidemic typhus. However, certain essential differences are of sufficient constancy to be worth noting.

1. The white matter of the cortex was involved in 4 of the 7 cases; the gray matter was spared in every instance. This distribution of lesions is directly divergent from the finding of nodules in the gray and none in the white matter of the cortex of both scrub and epidemic typhus.

2. Unlike the characteristically constant type of nodule of scrub and epidemic typhus, the parenchymal lesion of the brain of Rocky Mountain spotted fever is basically a microinfarct seen in a variety of stages. Although the capillaries are damaged, the vascular involvement is essentially of an entirely different order in spotted fever as compared to the other two. Herein, presumably, lies the basis for the differences in the lesions. Frequently, fairly large parenchymal arteries were appreciably altered by fibrinoid degeneration of their walls, swelling, hyperplasia, hyperchromasia and karyorrhexis of the endothelial cells, with thrombosis, and karyorrhexis of some nuclei included within the thrombi. These changes extended from the arteries to the minute capillaries. The vessels so involved might be surrounded by a few glial cells and various hematogenous mononuclear cells, but even about the damaged capillaries such proliferations only superficially simulated the nodules of scrub and epidemic typhus. Instead, there was seen along the affected vascular arborization, usually at the level of the arteriole, one or another stage of a microinfarct (Fig. 72). The lesion might vary from swollen, acidophilic, granular, and hyaline masses of myelin to a ragged, moth-eaten, frayed area about one to three times the size of a renal glomerulus. As a rule these foci attracted glial cells, including cytoplasmic astrocytes.

If the basic parenchymal lesion of these three diseases is compared with corresponding lesions of nonrickettsial protozoan diseases, the encephalitis of Rocky Mountain spotted fever and toxoplasmosis may be considered to be of a similar histologic order. In contrast, the en-

cephalitis of epidemic typhus and of scrub typhus may be grouped with that of Chagas' disease. In addition, the malarial "granulomas" of the brain simulate the "microinfarcts" of spotted fever.

GASTROINTESTINAL TRACT

Sections of gastrointestinal tract from 19 cases were examined. These included 16 sections of stomach, 8 of small intestine, and 5 of large intestine. In 1 case there was a diffuse infiltration of the wall of the stomach and ileum with lymphocytes, plasma cells, macrophages, and many polymorphonuclear leukocytes. In the remainder the infiltration was minimal and was characterized by small foci of perivascular mononuclear cells, particularly in the muscular layers. In 2 cases, there were small foci of thrombophlebitis in the stomach and colon. The findings in the gastrointestinal tracts of the cases of epidemic typhus and Rocky Mountain spotted fever were not significantly different.

GALLBLADDER

Sections of gallbladder were studied in 3 cases. One case showed nothing of significance. In the other 2 cases there was a remarkable, acute diffuse cholecystitis characterized by dense infiltrations of lymphocytes, plasma cells, and macrophages in one instance, and a predominance of neutrophilic and eosinophilic leukocytes in the other. In the latter case there was thrombophlebitis of a small subserosal vein. The gallbladders of 3 cases of epidemic typhus and 1 case of Rocky Mountain spotted fever showed only a few minimal interstitial infiltrations of mononuclear cells.

URINARY BLADDER AND PROSTATE

The urinary bladder was studied in 10 cases and the prostate in 11 cases of scrub typhus. Usually only scattered, small, perivascular foci of mononuclear cells were found. In 3 instances the concentration of cells in the mucosa warranted the diagnosis of low-grade cystitis. Six of 9 cases of epidemic typhus presented chronic cystitis and 5 of these were infected locally with ova of *Schistosoma haematobium*. In the 4 cases in which sections of prostate were examined, nothing of significance was found. Sections of urinary bladder and prostate from cases of Rocky Mountain spotted fever were not available.

THYROID

Sections of thyroid from 4 cases of scrub typhus, 11 cases of epidemic typhus, and 2 cases of Rocky Mountain spotted fever showed nothing remarkable.

SKELETAL MUSCLE

The sections of diaphragm in 2 cases of each of the three diseases revealed slight focal myositis. No vascular changes were demonstrable. However, in the sections of tongue from 18 cases of epidemic typhus myositis was present in all but 1 case. Vascular lesions, including necrotizing thrombophlebitis, thrombo-arteritis, and capillaritis, were prominent in epidemic typhus. Sections of tongue from cases of scrub typhus and spotted fever were not available.

BONE MARROW

The sections of vertebral bone marrow from 10 cases of scrub typhus, 19 cases of epidemic typhus, and 3 cases of Rocky Mountain spotted fever were similar and revealed a hyperplastic marrow. Phagocytosis of red and white blood cells by macrophages was observed frequently.

DISCUSSION

In integrating the histologic data, several points of divergence of our findings from those previously recorded become evident, and certain problems in pathogenesis are posed.

Eschar

Occurrence. One of the curious phenomena of the tsutsugamushi group of diseases is the occurrence, at the site bitten by the mite, of an eschar in most of the entities and its absence in others (*e.g.*, in Malayan rural typhus). This discrepancy occurs notwithstanding the presumed essential identity of the organisms, of the trombiculae, and indeed, of the diseases themselves. In the explanation of this phenomenon there must reside information of a fundamental nature concerning the selective tropism of arthropods for man, the effect of their venom, and the mechanism of spread of their infectious inoculum.

It is known that if the rickettsiae of tsutsugamushi disease are inoculated intracutaneously in animals, a grossly typical eschar is produced. However, the subcutaneous or intramuscular injection of the organisms does not provoke an eschar although the generalized infection follows.[14] Similar observations have been made in experimental fièvre boutonneuse, a form of spotted fever characterized, too, by a primary lesion or *tache noire*.[15] On the basis of these findings it has been suggested, without strong conviction, that the larval mites may perhaps be introducing the organisms at different levels of skin in various countries and in various peoples.[16] However, the solution is probably not so simple a matter as the length of the proboscis of the larval mite, which, in its entirety, measures hardly the thickness of

average epidermis. It is therefore unlikely that the proboscis extends below the dermis. Granted that the disease is transmitted through the skin by the trombicula, the alternative explanations of the local lesion appear to be: (1) that the rickettsiae are introduced directly into lymphatic vessels, a manipulation difficult to conceive; or (2) that there is a natural, local, cutaneous immunity in certain groups of people to the effect of the bites of mites just as there is to the bites of other arthropods. The latter viewpoint would appear more tenable. However, these are problems that await solution.

Pathogenicity of Eschar. Inasmuch as the noninfected trombicular larva may produce a lesion qualitatively not sharply different from the infected eschar, according to Kawamura,[17] the question arises as to whether the primary lesion is produced by the venom of the mite or by the rickettsiae injected. In favor of the interpretation that the secretion of the larva provokes the reaction are: (1) the histologic evidence from the examination of noninfected lesions produced by the *T. akamushi;*[17] (2) the experimental observations of the cutaneous effects of the emulsified larvae;[18] and (3) our observations of the analogous reactions produced by the secretion of ticks. On the other hand, there is convincing inferential evidence that the rickettsiae play a rôle in the reactions. In the first place, as stated, lesions that resemble eschars and *taches noires* have been produced by the injection of rickettsiae of tsutsugamushi disease and of fièvre boutonneuse. Secondly, there is evidence of a phlebitic reaction at the base of the eschars, away from the site of suppuration, that is identical with the phlebitic reaction in other organs and hence strongly indicates a response to rickettsiae or their products. Thirdly, we have observed the organisms in the eschar in one case, and the probability is great that they were present in other cases but not demonstrable by our methods. In other words, it appears reasonable to conclude that the reaction in the eschar is the summation of the effects of the rickettsiae *and* of the secretion of the mite. It is hoped that facilities will soon permit experimental studies of the rickettsial content of the eschars at various stages of the disease. It would be of further interest to determine the rapidity with which the organisms reach the regional lymph nodes.

Specificity of Typhus Nodule of Macule

The specificity of the typhus nodule of the macule has been repeatedly emphasized (Wolbach, Todd, and Palfrey,[19] Ceelen[20]). Such nodules are found not only in epidemic typhus and Rocky Mountain spotted fever but also in scrub typhus. However, we should prefer to qualify the impression of specificity to this extent: perivascular ac-

cumulations of mononuclear cells of this variety are found in the corium in many dermatoses; *e.g.*, lichen planus, parapsoriasis, toxic erythema. Moreover, the endothelial cells of the surrounded vessels in these dermatoses may be swollen and even hyperchromatic (Fig. 14). Unless the nodule is associated with thrombosis of the vessel, karyorrhexis of its endothelial or adjacent infiltrating cells, and granular or fibrinoid degeneration of its wall (Fig. 23), we should be reluctant to regard the lesion as specific. Moreover, diseases such as disseminated lupus erythematosus, periarteritis nodosa, erythema elevatum diutinum, and bacterial sepsis may present some of the vascular changes just mentioned, and accordingly deserve differential histologic consideration.

Lung

In over half of the cases of scrub typhus we noted various degrees of interstitial pneumonitis. In 13 per cent of the cases the reaction was intense (Fig. 27) and seemed to us indistinguishable from the pneumonitis of "Q" fever[8] (Fig. 36), from the pulmonary reaction seen occasionally associated with severe rheumatic carditis,[21] from the pneumonia of toxoplasmosis [22] (Fig. 29), and from the outspoken cases of so-called atypical or viral pneumonias.[23] Although we have failed to demonstrate the organisms in sections, there are reasons for believing that this reaction merits the designation "rickettsial pneumonia." Other observers have demonstrated the rickettsiae of tsutsugamushi disease in the alveoli and alveolar lining of experimental animals.[14] It is of inferential interest that the occasional, large hyperchromatic cell of the alveolar lining in the pneumonic lungs of scrub typhus (Fig. 28) has a direct counterpart in the large alveolar cell of the interstitial pneumonitis of toxoplasmosis, in which cell numerous parasites may be found (Fig. 29).

Slight to moderate degrees of interstitial pneumonitis were found in a few of our cases of epidemic typhus and in one case of Rocky Mountain spotted fever. We were unable to find descriptions of this finding in the observations of others, although the "interstitial changes" mentioned by Dawydowskie [24] may possibly represent the same lesion. In none of our cases did the intensity of the reaction approach that of the more florid cases of scrub typhus or of Lillie's cases of "Q" fever.[8]

Heart

One of the outstanding features of the cardiac status in each of the rickettsioses is the disparity between the severity of the myocarditis and the degree of cardiac insufficiency. The corollary to this impression is the apparent state of preservation of the myocardial fibers notwith-

standing the intensity of the interstitial infiltrate. However, we are not entirely certain that the muscle of the heart is as well preserved as a first evaluation might indicate. We are concerned by a type of fragmentation of myocardial fibers, especially those in relation to foci of edema and inflammation (Fig. 16). This finding is noted in each of the rickettsial diseases. It would seem hazardous to dismiss such changes as artifacts. It is acknowledged that patients who survive the illness do not show clinical evidence of cardiac involvement. Nevertheless, until post-mortem data are available on the state of such hearts years after recovery, the impression of complete clinical recovery might perhaps be tempered with these considerations: (1) Patients with a fine, scattered fibrosis of the myocardium may not manifest clinical evidence of cardiac damage; (2) Not all patients with scrub typhus develop a myocarditis (Table II), and it is therefore conceivable that a portion of the survivors may belong in this category; and (3) Adequate post-mortem studies of the myocardium months or years after recovery are not yet available. The fact is that it is not known whether or not the myocardium is restituted to *status quo ante*.

Sickling

Apparent sickling of red blood cells was observed in the sections of one or more organs, usually the kidney, in 13 per cent of the cases of scrub typhus, in 35 per cent of the cases of epidemic typhus (Figs. 40 and 84), and in 1 of the 12 cases of Rocky Mountain spotted fever. The patients with epidemic typhus were all native Egyptians. We do not have any information on the incidence of sickling in Egyptians although studies of the West African native reveal an average incidence of 20 per cent.[25] The evidence of the sickling in scrub typhus and spotted fever was observed in isolated organs of patients, all of whom were recorded as being of the white race. No data are available on the *in vivo* occurrence of sickling in patients with typhus fever. If this slight degree of sickling of red blood cells truly represents a latent sickling tendency, then the incidence seems remarkably high.[26] Such focal sickling of red blood cells is seen occasionally in routine autopsy material of patients dying of a variety of causes. The impression is generally held that the phenomenon represents a post-mortem packing and distortion of cells. However, it must be noted that individual, isolated cells in these foci assume a sickled shape (Fig. 40). The pathogenesis of such distortion of erythrocytes cannot be considered settled. Therefore, because the significance of the observation is not clear, and because of the possibility, at least, that it may reflect local anoxia, the finding is deemed worthy of record and comment.

Rickettsiae in Tissues

There was available to us only a single smear from cases of scrub typhus. This one smear was of spleen and with the Giemsa stain (Wolbach modification [27]) numerous rickettsiae were found (Fig. 44). A systematic search for rickettsiae in sections was made in only 6 cases of scrub typhus, the tissues of which were fixed in Regaud's solution. Intracytoplasmic endothelial bodies, which we are confident are rickettsiae, were found in only one section—that of an eschar. We were, however, unable to obtain a satisfactory photograph for purposes of demonstration in this paper. It is clear that rickettsiae are discovered with far greater ease in the sections of Rocky Mountain spotted fever and louse-borne typhus, than in tsutsugamushi disease. Of importance in facilitating the detection of organisms is the cutting of sections of not more than 4 μ in thickness, as well as proper fixation and staining.[27] At best, the unmasking of rickettsiae in the tissues of scrub typhus is currently an unreliable procedure.

Kidney

Apart from focal interstitial nephritis, cloudy swelling and vacuolization of the tubular epithelium, and occasional vascular alterations, the kidneys of the rickettsioses have been described in the literature as showing few significant changes. The glomeruli are usually dismissed as being essentially normal. For example, Kawamura,[17] in his exceedingly able and comprehensive discussion of tsutsugamushi disease, did not mention glomerular damage. Kouwenaar,[28] in his study of Sumatran mite typhus, stated that "for the most part the glomeruli are intact; occasionally there is definite capsular adhesion." In their classic study of epidemic typhus, Wolbach, Todd, and Palfrey [19] found glomerulonephritis in 1 of 37 cases, and intracapillary proliferation in 9 others. Ceelen [20] found no glomerular alteration in his cases of epidemic typhus. Munk [29] mentioned the finding of "Infektnephritis" in instances of epidemic typhus in which hematuria was present without significant glomerular alteration. In Rocky Mountain spotted fever, Wolbach [27] found essentially normal glomeruli except for an increased cellularity in 1 of the 5 cases. In Lillie's series of cases of spotted fever,[13] the glomeruli were not remarkable. Several conspicuous exceptions are found in the literature and these are in connection with *epidemic typhus*. Caffarena [30] found acute diffuse glomerulonephritis in 67.5 per cent of his cases. Schopper [31] observed swelling and hyperplasia of glomerular endothelial cells and suggested that his cases might represent the earliest hemorrhagic phase of glomerulonephritis. Dawydowskie [24] noted a focal destructive glomerulitis in one-third of his

series of cases of louse-borne typhus. Wetzel,[32] in describing a case of epidemic typhus with glomerulonephritis, insisted that too little emphasis was being paid to this lesion.

The interpretation of the glomerular changes in the current material approximates the conclusions of Caffarena.[30] We found acute diffuse glomerulonephritis* in 30 per cent of the cases of scrub typhus, 78 per cent of epidemic typhus, and 50 per cent of Rocky Mountain spotted fever (Figs. 52 to 56, and 83). Our data did not permit of a systematic correlation of the histologic changes with alterations in the chemical constituents of the blood. It is hoped that detailed correlative studies will soon be forthcoming. The glomerular lesions in the three rickettsial diseases are basically similar. They represent the intracapillary form of acute diffuse glomerulonephritis, characterized by swelling and hyperplasia of endothelial cells, normal or slightly thickened, sometimes duplicated, occasionally hyalinized, capillary basement membranes, and marked ischemia of the tufts. Intracapillary hyaline fibers[32a] were not present, but in our experience absence of such fibers does not preclude the existence of glomerulonephritis with impairment of renal function. The high incidence of acute diffuse glomerulonephritis (78 per cent) in the current series of cases of epidemic typhus is in harmony with the recent observation made by Yeomans, Snyder, Murray, Zarafonetis, and Ecke[33] who noted clinically that typhus fever frequently produced severe impairment of renal function.

Pathogenesis of Glomerulonephritis. If the existence of glomerulonephritis is granted, two questions arise: (1) Is the glomerulonephritis, *i.e.*, the injury to the glomerular capillaries, due to the direct action of the rickettsiae? (2) What are the chances of restoration of the glomeruli to normal? We have not found organisms in the glomeruli in any of the typhus fevers, nor do we know of any such observation. It seems unreasonable to assume that organisms would lodge directly in the endothelium of glomerular capillaries and provoke a fairly uniform response throughout the kidney. The damage to vessels in other organs in which rickettsiae are found is characteristically varied in intensity. Therefore the glomerular alteration is regarded as a remote effect of the rickettsiae—either hyperergic or toxic—in much the same sense that acute diffuse glomerulonephritis following scarlet fever is attributed to the distant or secondary effects of *Streptococcus haemolyticus*. As we understand them, this is essentially the view of Julliard and Henaff.[34]

Restitution of Glomeruli. Notwithstanding the development of glomerulonephritis, it appears that the chances for anatomic restora-

* Although we are confident that these cases represent an early acute diffuse glomerulonephritis, we are aware that some pathologists may not concur with this interpretation in all instances.

tion of the glomeruli are encouraging. Disturbance in the basic architecture of the glomeruli is infrequent. The basement membranes are usually not appreciably affected, notwithstanding the serious endothelial changes and the resulting ischemia of the capillaries. The cloudy swelling and often marked hydropic vacuolization of the proximal convoluted tubules are entirely reversible. The focal interstitial inflammation that is usually present will either resolve or cause cicatrization, depending on its severity, but it is not likely to constitute a source of significant impairment of renal function in the future. Finally, an analogy is afforded by the resolution of acute diffuse glomerulonephritis that undoubtedly occurs in many cases following recovery from bacterial infections.[35]

Circulatory Failure

There has been considerable discussion in the literature regarding the pathogenesis of the circulatory collapse of patients with typhus fevers. The rôles of the myocardium and of the central nervous system have been emphasized. However, in the rickettsioses, no less than in many instances of bacterial sepsis, the decisive, terminating clinical effects are a product of the disturbances of more than one organ. In view of the histologic changes, it would seem justified to consider the contributory effects particularly of the heart, the central nervous system, the lungs, the kidneys, and the adrenals. The heart has been discussed. The findings in the central nervous system are impressive, but we have not been able to study the vital centers systematically. Therefore we do not have the detailed data that would be necessary for an evaluation of this phase of the problem. From studies of the nervous system in epidemic typhus,[36] and from our own inadequate observations, there is reason to believe that the nervous system reacts to a degree comparable to that seen in other organs.

Circulatory collapse occurs commonly without significant involvement of the lungs, but it is interesting that there is more or less parallelism between the intensity of the interstitial pneumonitis and of the myocarditis. Surely, pneumonitis, when severe, contributes to the circulatory load. The kidneys, as stated, are frequently involved and undoubtedly contribute to the hemodynamic burden, and to the death of the patient, but it is to be emphasized that circulatory collapse may occur without significant, histologically evident, renal damage. The expression "histologically evident" is used advisedly because of the possibility that the adrenal gland, for example, may mediate its vital control of the electrolytes of the blood through the kidneys without provoking microscopically visible clues.[37] The adrenal gland is mentioned at this point and in this connection because we have observed

the same type of "tubular degeneration" of the cortex in rickettsial disease as Rich [10] has recently noted in cases of bacterial sepsis (Fig. 49). Rich suggested that this degeneration may be related to the production of shock in septicemias. It is pertinent to recall that less than a decade ago, considerable routine importance was attached to the rôle of the adrenal cortex in the genesis of shock in infectious states. At that time, adrenal cortical therapy was regarded as effective by some [38, 39] and ineffective by others.[40] Possibly the lack of convincing histologic support discouraged prolonged interest, which may now be renewed with the impetus of Rich's observation. Undoubtedly, this change in the cortical cells is regarded by many as post-mortem degeneration, an opinion strengthened, perhaps, by the susceptibility of the inner zone to post-mortem liquefaction. However, the "tubular degeneration" to which we refer occurs in the outer zones and we are convinced that the lesion is not an artifact, whatever its physiologic implications may be. The fact is that shock with vasomotor atony and collapse, hypochloremia, azotemia, and changes in blood volume are present in the severe cases of rickettsial infection. The further fact is that these are precisely the changes associated with adrenal insufficiency. The data at hand do not permit drawing a complete comparison, but the parallel is, at the least, suggestive. We should be reluctant to adopt the mechanistic explanation for the vascular atony and increased permeability that occur in epidemic typhus and which have been attributed to actual damage to the vessels produced directly by the rickettsiae.[41] To be sure, such intrinsic vascular damage is often evident in louse-borne typhus, but it is usually lacking in scrub typhus, that is, unless it is inferred from the perivascular infiltrate that the vessel concerned has been altered, an inference by no means necessarily justified. Moreover, notwithstanding the difference in morphologic evidence of vascular injury, the severity of vasomotor collapse appears just as striking in scrub typhus as in epidemic typhus. It would, therefore, seem more likely that this profound hematic and vasomotor disturbance is mediated by a universally acting, humoral mechanism rather than by local organic changes of various stages and degrees. The rôle of the adrenal cortex in this physiologic upheaval and the therapeutic trial of cortical extract is again suggested for consideration.

The Cellular Infiltrate

The quality of the cellular infiltrate in all of the organs of the rickettsioses is basically similar and consists essentially of lymphocytes, plasma cells, and large basophilic as well as acidophilic macrophages. The large basophilic macrophage has repeatedly been singled

out for comment in discussions of the rickettsial diseases. It has been variously labelled Türk cell, large lymphocyte, endothelial phagocyte, etc. It is discussed here because it may throw light on one aspect of the pathogenesis of the lesions in the typhus fevers.

These cells are produced in abundance in the spleen following the repeated injection of foreign serum; [42] indeed, the cytologic picture of such spleens simulates that in typhus fever. The evidence in both experimental and autopsy material suggests that the cells belong to the lymphatic series. Transitions from the small lymphocyte, with basophilic cytoplasm and pachychromatic nucleus, to the large basophilic macrophage and thence to the leptochromatic, acidophilic macrophage have been observed by numerous investigators (Huebschmann,[43] Tschaschin,[44] Maximow,[45] Bloom,[46] Taliaferro and Mulligan,[47] and Kolouch [48]). In other words, the *hematogenous* macrophage may become indistinguishable from the *histogenous* macrophage. Moreover, this large basophilic macrophage, or, "acute splenic tumor cell," has been shown most graphically by Rich, Lewis, and Wintrobe [42] to have the characteristics of a lymphoblast, an opinion, as they stated, which was previously expressed by Huebschmann.[43] It is of considerable pathogenetic interest that this cell is found in abundance in allergic reactions.[42] Moreover, this evolution of the lymphocyte helps to explain the concentration of various stages of the series of mononuclear cells in veins of many organs (Fig. 57). Apparently, these large basophilic macrophages have been observed by Rabinowitsch to the extent of 0.5 to 10 per cent of the white blood cells in cases of epidemic typhus.[49] It is suggested that detailed studies of these cells in the typhus fevers be made from the peripheral blood as well as from smears of organs at autopsy. Information concerning the time of appearance and disappearance of these cells from the blood might be revealing.

Hyperergy

While we are aware of the burden that is prone to be attached to an interpretation of the genesis of a histologic change in terms of allergy, nevertheless, certain alterative changes in the rickettsioses are so striking as to merit discussion. These include: (1) necrosis of lymph nodes and spleen, simulating responses to known allergens, (2) the predominance of plasma cells and especially of large basophilic macrophages, which are known to participate in allergic reactions [42] (eosinophilic leukocytes were inconspicuous), (3) the glomerulonephritis, (4) the mononuclear cell infiltration and edema of the mural and valvular endocardium as well as of the intima of arteries (Fig. 19). This reaction is seen not only in sepsis generally, but following sensitization to

serum.[7] (5) Finally, there is the pronounced fibrinoid degeneration of the interstitial collagen of the myocardium (Fig. 17), of the vessels (Fig. 22), and of the perivascular tissue (Fig. 25). This type of alteration has long been identified with hyperergic responses.[50-52] The same type of fibrinoid alteration is observed in periarteritis nodosa, in disseminated lupus erythematosus, and in the vascular damage following sensitization to the sulfonamides.[53,54] In short, these various reactions, considered together, appear to us to present strongly presumptive histologic evidence of "altered tissue reactivity" or one phase of allergy. In this regard, it may be recalled that the phenomena of allergy apply to the rickettsiae as well as to bacteria (Mudd [55]).

Pathologic Concept of the Rickettsioses

From the pathologic point of view, the rickettsioses have long been regarded as a form of diffuse vascular disease. Surely, this impression is almost inescapable after a study of epidemic typhus and spotted fever. However, the histology of scrub typhus may perhaps warrant a change in the direction of emphasis. Although focal, more or less bland thrombophlebitis in scrub typhus is not uncommon, actual arteritis occurs rarely, and, in our series, was never of the fibrinoid variety seen in louse-borne or tick-borne typhus. Moreover, the arteritis of scrub typhus does not seem to be a lesion *sui generis*, but, rather, appears to be secondary to an extension of the periarterial infiltrate into the wall. This interpretation was made previously by Kouwenaar.[28] Yet, notwithstanding the disparity in the histologic evidences of vascular damage, there are basic clinical, etiologic, and, in many respects, immunologic similarities between scrub typhus and the other rickettsioses. Therefore, perhaps a re-evaluation of the significance of the pathologic changes is in order. A close analogy to this problem is found in a nonrickettsial disease—acute disseminated lupus erythematosus (Libman-Sacks disease). The prominence of the degeneration of vessels in many organs led initially to the concept that this entity was a diffuse vascular disease. However, further studies prompted a broader concept; namely, that disseminated lupus erythematosus was in effect a disturbance of collagen, be it of a vessel, a cardiac valve, or a serous membrane. Moreover, the histologic and clinical pictures were such as to suggest a hyperergic reaction.[52, 56, 57] The analogy may be extended by reference to periarteritis nodosa and to the arteritis that follows administration of sulfonamides. In other words, in the over-all view of the pathologist, the more remote, possibly hyperergic effects of the rickettsiae—the effects on the adrenal gland, on the glomeruli, and on the production of interstitial inflammation—assume

more importance than the direct damage wrought by the localization of the rickettsiae.

Summary and Conclusions

1. The histologic preparations and protocols of 78 cases of scrub typhus (tsutsugamushi disease), 24 cases of epidemic (louse-borne) typhus, 12 cases of Rocky Mountain spotted fever, and the sections of lungs of 2 cases of American "Q" fever were studied.

2. The primary lesion, or eschar, is considered to be provoked by the combined action of the secretion of the larval mite and the inoculated rickettsiae. It is suggested that the absence of the eschar in certain instances of scrub typhus may be due to variations in cutaneous immunity.

3. Interstitial pneumonitis of a marked degree is common in scrub typhus in contrast with epidemic typhus and Rocky Mountain spotted fever. The histologic picture of the interstitial pneumonitis of scrub typhus is indistinguishable from that of "Q" fever, rheumatic fever, toxoplasmosis, and viral pneumonia.

4. It is concluded that the amount of hepatic damage as noted histologically does not warrant the presumption that hypoproteinemia is due to hepatic insufficiency.

5. Early, acute, diffuse glomerulonephritis is common in scrub typhus, epidemic typhus, and Rocky Mountain spotted fever. The indirect rôle of the rickettsiae in the pathogenesis of the glomerulonephritis is indicated.

6. The focal encephalitis or nodule of scrub typhus is qualitatively similar to that of epidemic typhus and is in contrast to the "microinfarct" of Rocky Mountain spotted fever. The nodules of scrub typhus and epidemic typhus are practically limited to the gray matter, whereas the encephalitis of spotted fever involves the white matter preponderantly.

7. Contrary to the generally held impression, there is a sparsity of histologically evident vascular damage in scrub typhus. Arteritis is exceedingly slight in scrub typhus in contrast with epidemic typhus and Rocky Mountain spotted fever. Accordingly, it is suggested that the designation "diffuse vasculitis" when applied to scrub typhus represents an oversimplification not justified by the morphologic evidence.

8. It is concluded that the peripheral circulatory failure in patients with rickettsial diseases is a complex phenomenon which cannot be explained solely on the basis of morphologic damage of vessels. The contributory rôle of the adrenal gland in the circulatory failure is suggested.

9. The evidence of lymphoblastic origin for the cells characterizing the interstitial infiltrate is presented. The identification of the large "basophilic macrophage" with the "acute splenic tumor cell" is suggested and the evidence pointing toward the association of these cells with an allergic response is given.

10. Reasons are presented for regarding the rickettsial diseases from a broader pathologic point of view than that of diffuse vascular diseases. Emphasis is placed on the importance of the indirect, possibly toxic, but more likely hyperergic effects of the rickettsiae, on the basis of certain histologic responses which are regarded as strongly presumptive evidence of the action of allergens. These responses include fibrinoid degeneration of collagen, the necrosis of lymph nodes and spleen, the predominance of the basophilic macrophage and associated cells, and the acute diffuse glomerulonephritis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 99

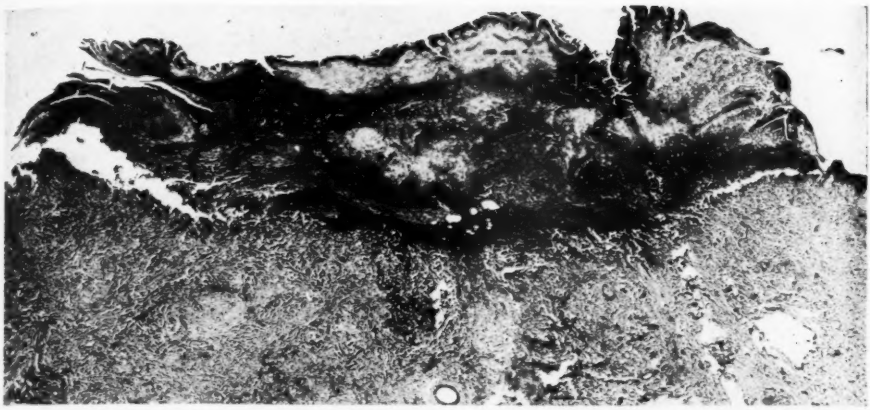
FIG. 1. Acc. 101690. Eschar of scrub typhus removed at autopsy. Duration of illness, 17 days. The superficial mass of necrotic epidermis, karyorrhectic cells, blood, serum, and bacteria represents the black crust that was about to be sloughed. Hematoxylin and eosin stain. × 20. Neg. 83436.

FIG. 2. Acc. 112440. Eschar of scrub typhus removed at autopsy. Duration of illness, 15 days. The residual epidermis at the base of the lesion indicates the initial intra-epidermal location of the pustule. Hematoxylin and eosin stain. × 50. Neg. 83399.

FIG. 3. Acc. 112440. Higher magnification of one of a series of sections from the lesion in Figure 2. The amorphous nature of the pustular contents, the distortion of the inflammatory cells, and the irregular acanthosis may be seen. Hematoxylin and eosin stain. × 145. Neg. 82918.

FIG. 4. Acc. 101690. Section from angle of eschar showing the intra-epidermal position of the pustule, its parakeratotic roof, the peripheral acanthosis, and vacuolization of the cells of the rete malpighii. Hematoxylin and eosin stain. × 145. Neg. 83153.

FIG. 5. Acc. 101690. Section of epidermis at the periphery of eschar showing parakeratosis, acanthosis, edema, hyperchromatism, and disturbance in polarity of cells of basal layer and lower stratum spinosum. Hematoxylin and eosin stain. × 500. Neg. 77500.



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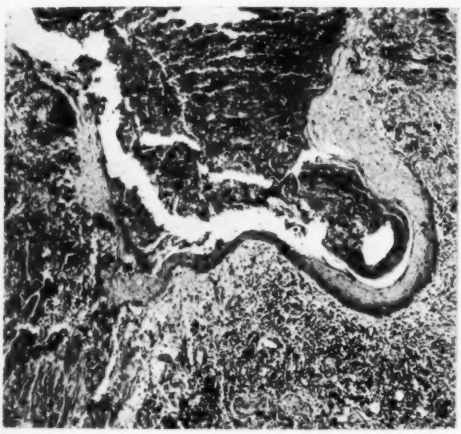

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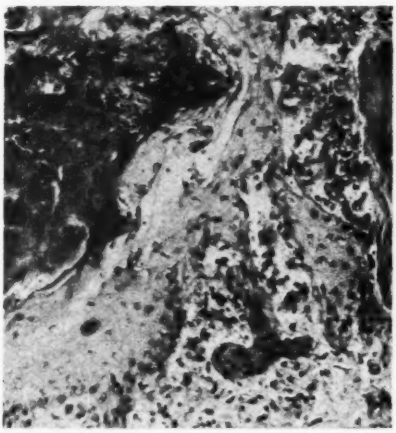

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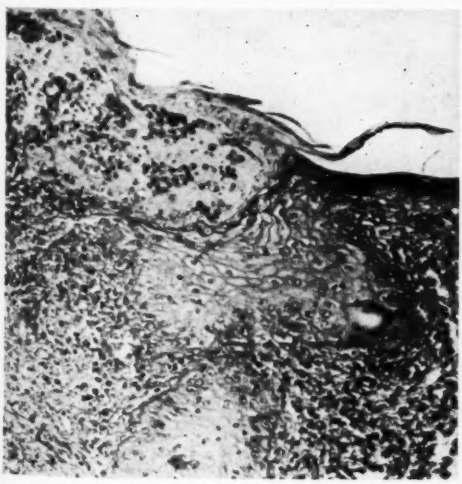

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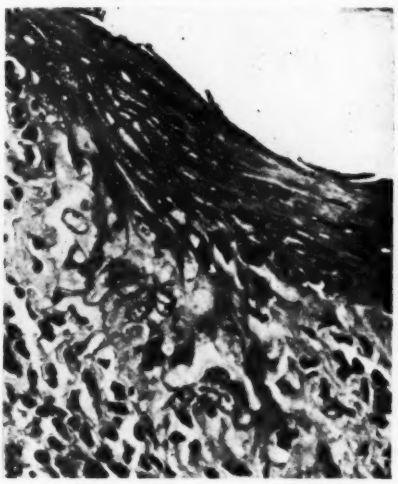

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Allen and Spitz

Comparative Study of Scrub Typhus

PLATE 100

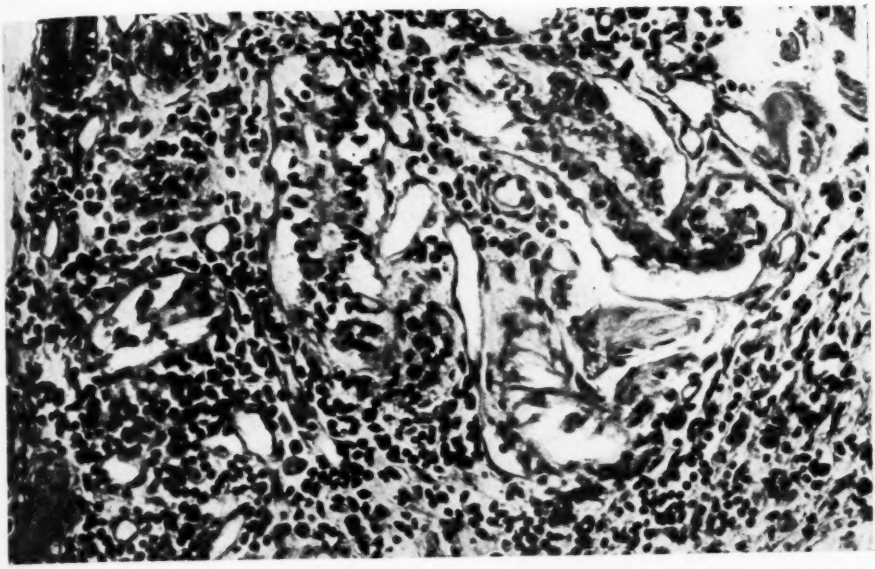
FIG. 6. Acc. 112440. Section of mid-corium shows the mononuclear character of the infiltrate and the localization about the coils of sweat glands. Hematoxylin and eosin stain. ×280. Neg. 83144.

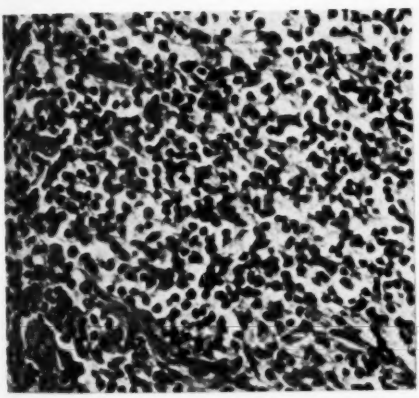
FIG. 7. Acc. 102989. The infiltrate in this section of skin produced by a sterile tick bite matches in quality that of the eschar except for the abundant eosinophilic leukocytes in the former. Hematoxylin and eosin stain. × 280. Neg. 83150.

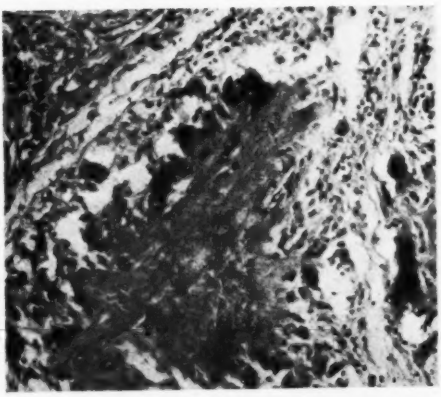
FIG. 8. Acc. 112440. Foreign body reaction in the corium of an eschar. A small fragment of mite, not apparent in this section, was found at the periphery of this lesion. Hematoxylin and eosin stain. × 120. Neg. 83143.

FIG. 9. Acc. 101690. Section taken from the lower reticular layer of the corium, away from areas of suppuration, showing mononuclear infiltration of the intima. This reaction, and that shown in Figure 10, represent the type of phlebitis found in other organs and is presumed to be due to the effect of rickettsia rather than to a nonspecific contiguous inflammation. Hematoxylin and eosin stain. × 145. Neg. 82921.

FIG. 10. Acc. 101690. Section showing phlebitis at junction of dermis and hypodermis of eschar. The granular type of intimal degeneration and the various stages in the formation of intimal verrucae may be noted. Hematoxylin and eosin stain. × 145. Neg. 83152.







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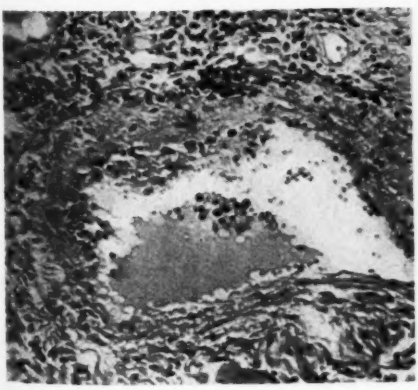

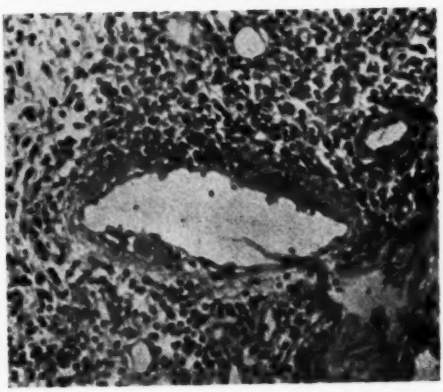

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Plate 101

Fig. 11. Acc. 112281. Macule of scrub typhus. Duration of rash, 7 days. There is a perivascular infiltration of mononuclear cells forming the equivalent of the "typhus nodule." Hematoxylin and eosin stain. × 145. Neg. 82911.

Fig. 12. Acc. 112525. Macule of scrub typhus. Duration of rash, 7 days. Of note is the edema of the papillary layer, the platelet thrombus, and the swelling and hyperchromasia of the endothelial cells of a venule. Hematoxylin and eosin stain. × 230. Neg. 82912.

Fig. 13. Acc. 105720-8A. Macule of epidemic typhus. Duration of rash, 13 days. The eccentrically perivascular "typhus nodule" and the thrombophlebitis are illustrated. Hematoxylin and eosin stain. × 230. Neg. 82913.

Fig. 14. Acc. 119287. Section of dermis from a case of lichen planus showing perivascular infiltration of lymphocytes, plasma cells, and macrophages as well as endothelial swelling. This nonspecific lesion simulates the "typhus nodule" but lacks endothelial karyorrhexis and thrombosis. Hematoxylin and eosin stain. × 500. Neg. 82970.

Fig. 15. Acc. 112435. Macule of scrub typhus. Duration of rash, 8 days. This photograph illustrates a type of degeneration of sweat glands seen not only in the various typhus fevers but in many dermatoses. Hematoxylin and eosin stain. × 120. Neg. 83159.

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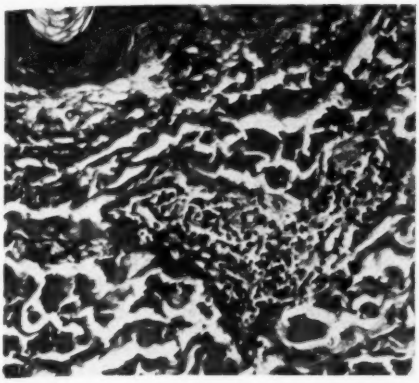

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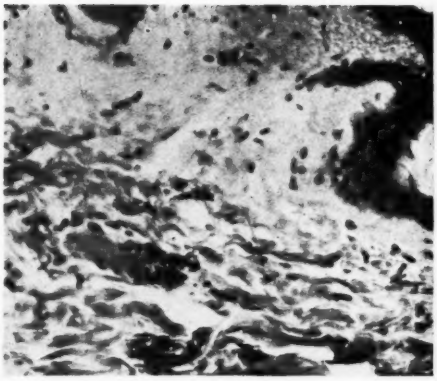

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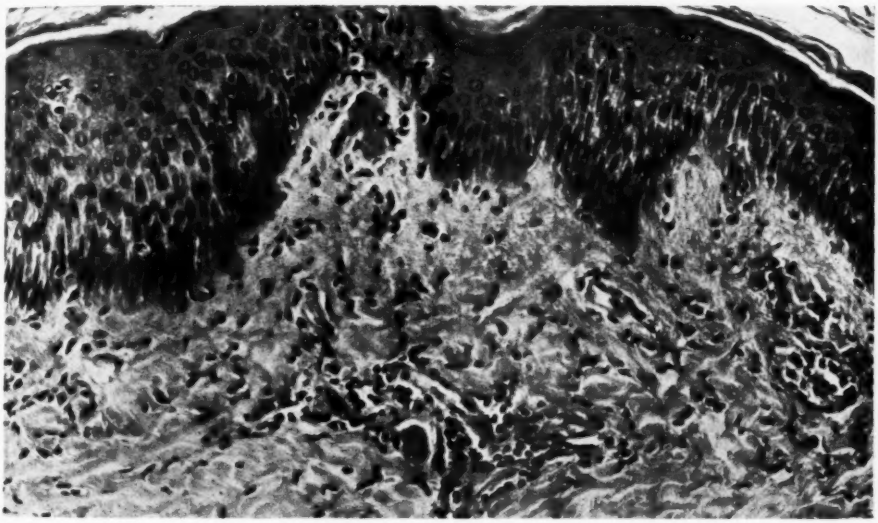

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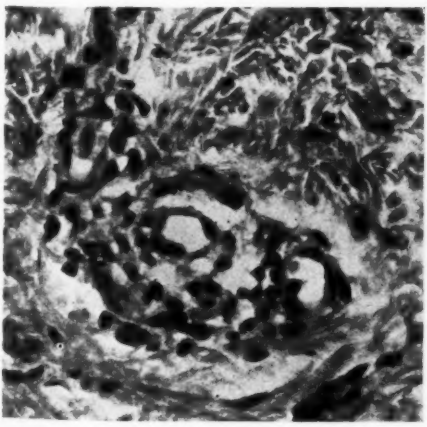

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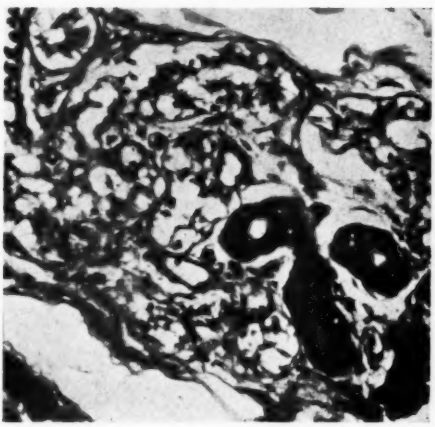

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PLATE 102

FIG. 16. Acc. 112440. Interstitial myocarditis of scrub typhus, showing (1) the interfibrous distribution, (2) the various types or stages of development of mononuclear cells, (3) the interstitial edema, and (4) the apparent degenerative changes in the muscle fibers. Hematoxylin and eosin stain. × 500. Neg. 83149.

FIG. 17. Acc. 112451. Interstitial myocarditis of scrub typhus with fibrinoid degeneration of collagen that strikingly simulates the interstitial lesion of the myocardium of disseminated lupus erythematosus (Fig. 18). Hematoxylin and eosin stain. × 750. Neg. 83147.

FIG. 18. Acc. 87549. Interstitial myocarditis of disseminated lupus erythematosus showing the type of fibrinoid degeneration almost pathognomonic of this disease. Hematoxylin and eosin stain. × 375. Neg. 83148.

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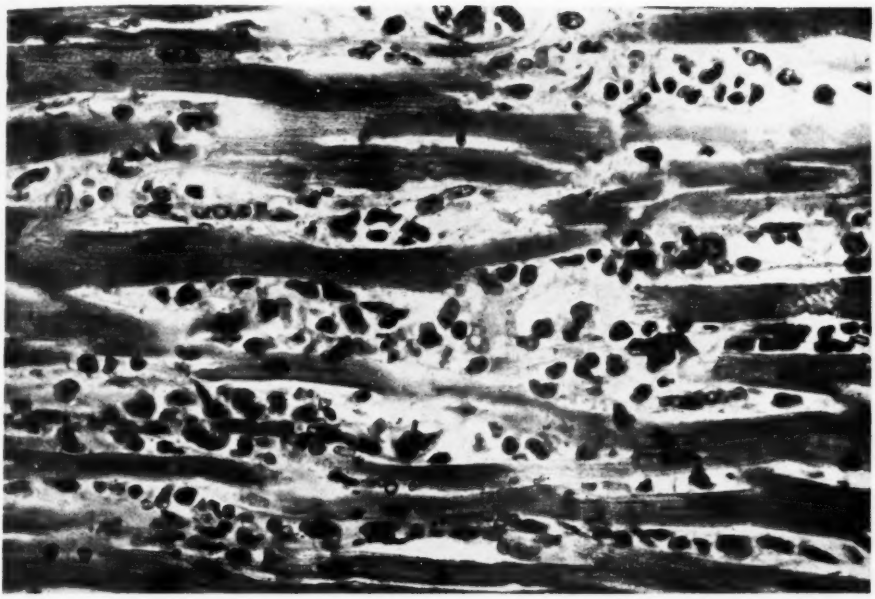

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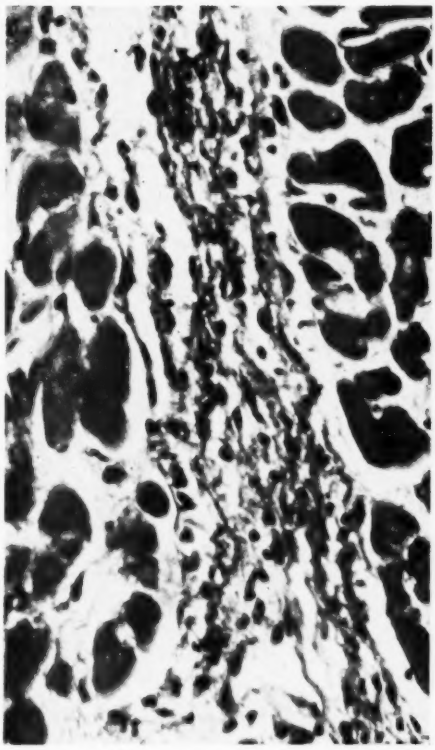


Allen and Spitz Comparative Study of Scrub Typhus

PLATE 103

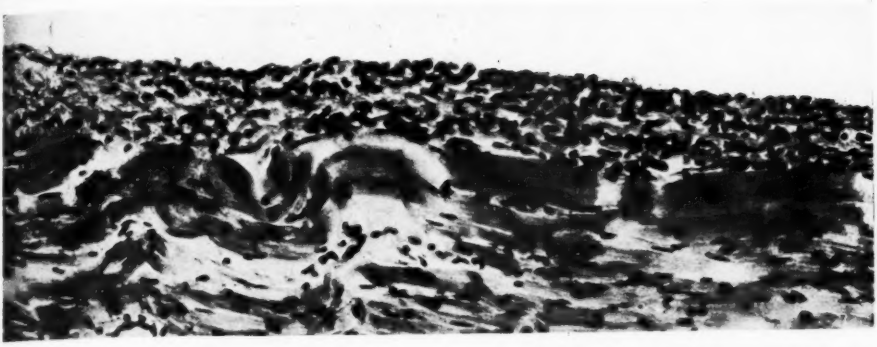
FIG. 19. Acc. 112281. Heart of scrub typhus showing marked infiltration of mural endocardium by various mononuclear cells. Similar infiltrations occur beneath endothelium of valves, coronary arteries, and aorta. Hematoxylin and eosin stain. × 250. Neg. 83016.

FIG. 20. Acc. 112451. Ischemic type of degeneration of myocardium of scrub typhus. Hematoxylin and eosin stain. × 230. Neg. 82900.

FIG. 21. Acc. 112525. Intramyocardial neuritis in scrub typhus. Hematoxylin and eosin stain. × 280. Neg. 82924.

FIG. 22. Acc. 105720-22A. A lesion of the type found in periarteritis nodosa in the myocardium of a patient with epidemic typhus. No eosinophilic leukocytes are present. Hematoxylin and eosin stain. × 230. Neg. 80318.

FIG. 23. Acc. 105720-21A. "Typhus nodule" of myocardium in epidemic typhus, showing hyperchromasia and swelling of endothelium of arteriole, granular degeneration of its wall, and karyorrhexis of cells. Hematoxylin and eosin stain. × 600. Neg. 78554.



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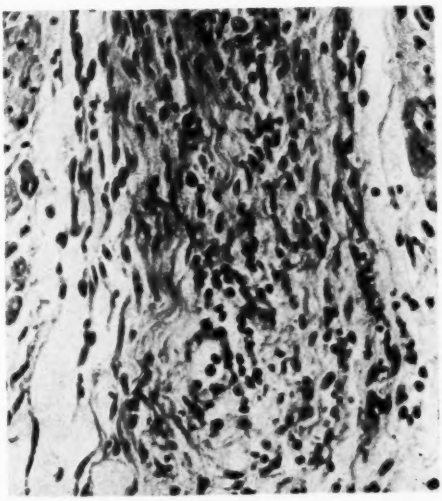

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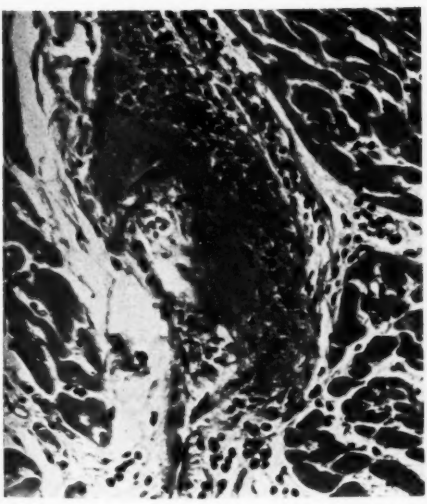

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Plate 104

Fig. 24. Acc. 94537. Interstitial infiltrate of myocardium in scrub typhus showing lymphocytes, plasma cells, basophilic macrophages, and several Anitschkow myocytes. Hematoxylin and eosin stain. × 705. Neg. 82932.

Fig. 25. Acc. 105720-22A. Epicardium of louse-borne typhus showing periarterial fibrinoid change, again of the type seen in disseminated lupus erythematosus. Hematoxylin and eosin stain. × 250. Neg. 83195.

Fig. 26. Acc. 113898. Hemorrhagic tracheitis in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82920.

Fig. 27. Acc. 113446. Interstitial pneumonitis of scrub typhus showing edema and mononuclear cell infiltration of septa and alveoli. Hematoxylin and eosin stain. × 120. Neg. 82723.

Fig. 28. Acc. 111595. Interstitial pneumonitis of scrub typhus showing not only the septal involvement but particularly the enlarged, hyperchromatic alveolar "epithelial" cells. Hematoxylin and eosin stain. × 500. Neg. 82730.

Fig. 29. Acc. 97029. Interstitial pneumonitis of toxoplasmosis showing a picture similar to Figure 28. The protozoan parasites are present in the largest alveolar lining cell included in this photomicrograph. Hematoxylin and eosin stain. × 330. (Material obtained through the courtesy of Dr. Henry Pinkerton, St. Louis University, School of Medicine.) Neg. 82904.

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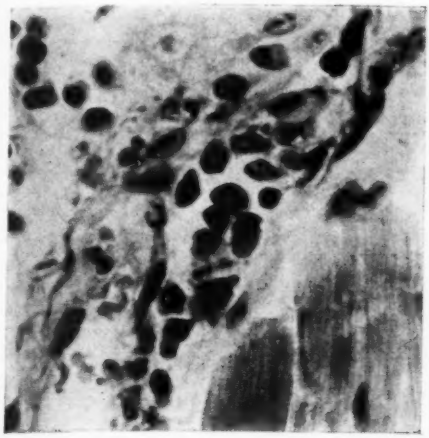

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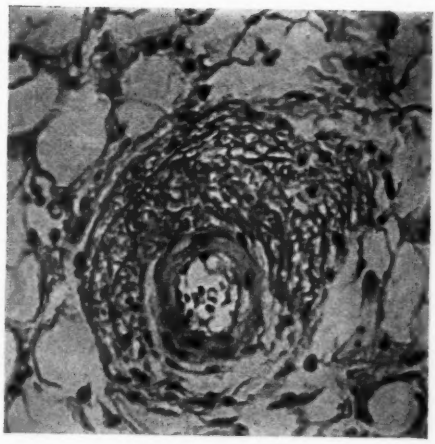

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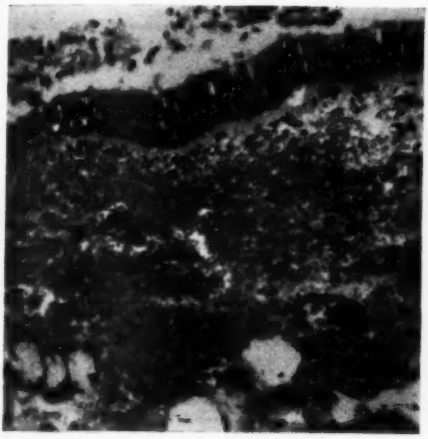

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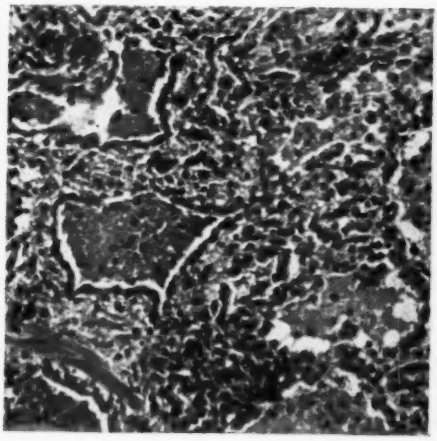

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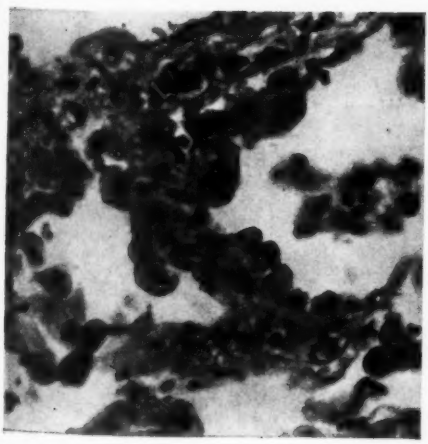

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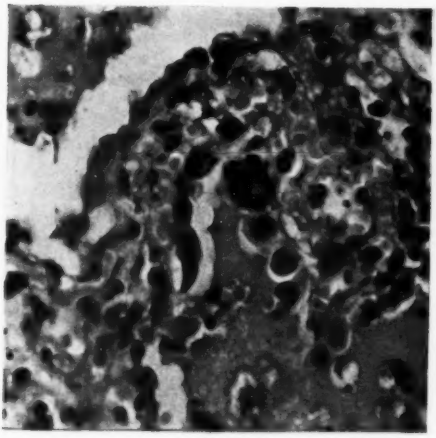


Plate 105

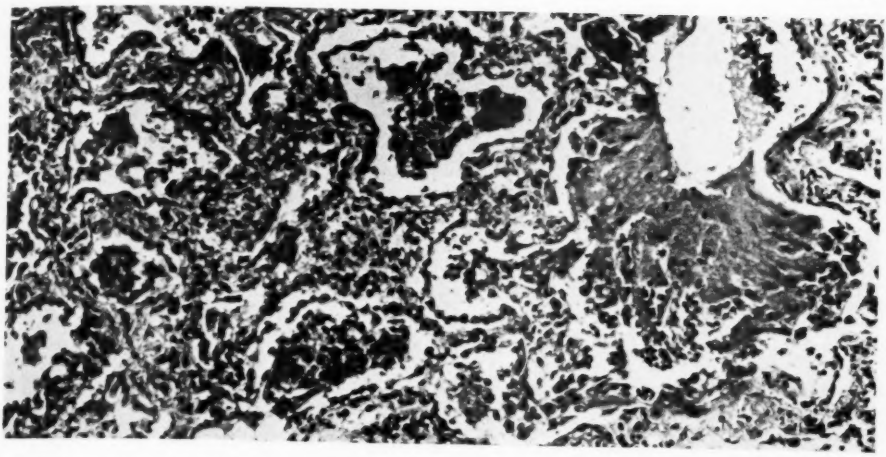
Fig. 30. Acc. 112304. Interstitial pneumonitis of scrub typhus showing (1) septal edema and mononuclear cell infiltration, (2) macrophages and partially organized alveolar exudate, (3) prominent alveolar lining. Hematoxylin and eosin stain. × 145. Neg. 82718.

Fig. 31. Acc. 112240. Interstitial pneumonitis of scrub typhus illustrating the septal edema and relative ischemia, as well as the hemorrhagic exudate. Hematoxylin and eosin stain. × 230. Neg. 82724.

Fig. 32. Acc. 112240. Interstitial pneumonitis of scrub typhus showing edema and mononuclear cell infiltration of an interlobar septum. The alveolar lining is prominent. Hematoxylin and eosin stain. × 120. Neg. 82729.

Fig. 33. Acc. 94655. Phlebitis in lung in case of scrub typhus, showing characteristic intimal mound of mononuclear cells. This type of phlebitis is seen commonly in the kidneys of scrub typhus. Hematoxylin and eosin stain. × 120. Neg. 82728.

Fig. 34. Acc. 112261. Pleuritis accompanying interstitial pneumonitis of scrub typhus. The mononuclear cells suggest a mesothelial origin. Hematoxylin and eosin stain. × 145. Neg. 82719.



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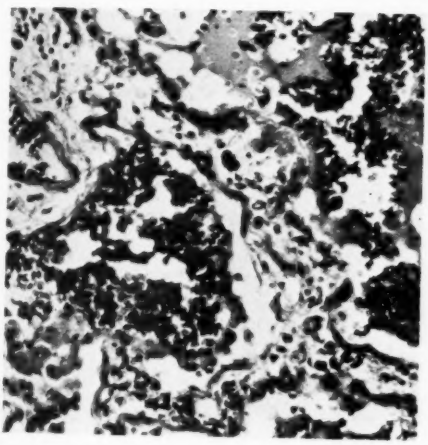

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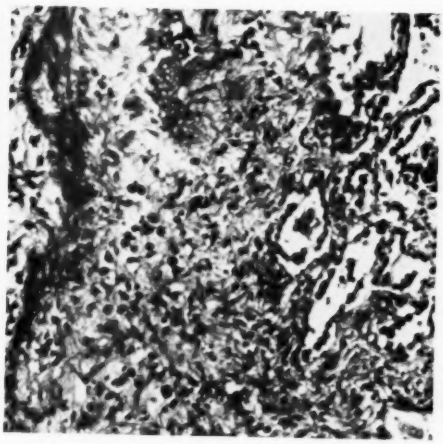

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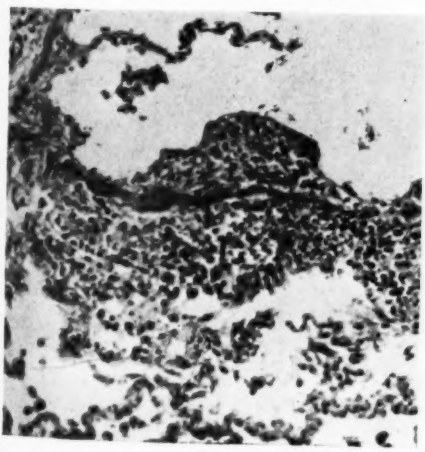

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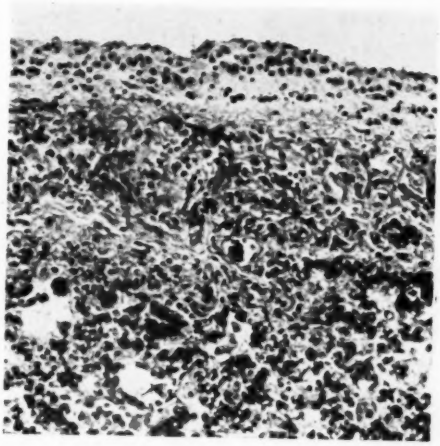

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PLATE 106

FIG. 35. Acc. 105720-14A. Interstitial pneumonitis in a case of epidemic typhus. Hematoxylin and eosin stain. × 115. Neg. 83188.

FIG. 36. Acc. 104973. Interstitial pneumonitis of American "Q" fever, with marked bronchiolitis and mononuclear cell infiltration of the septa and alveoli. This pneumonitis is similar to that observed in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82975.

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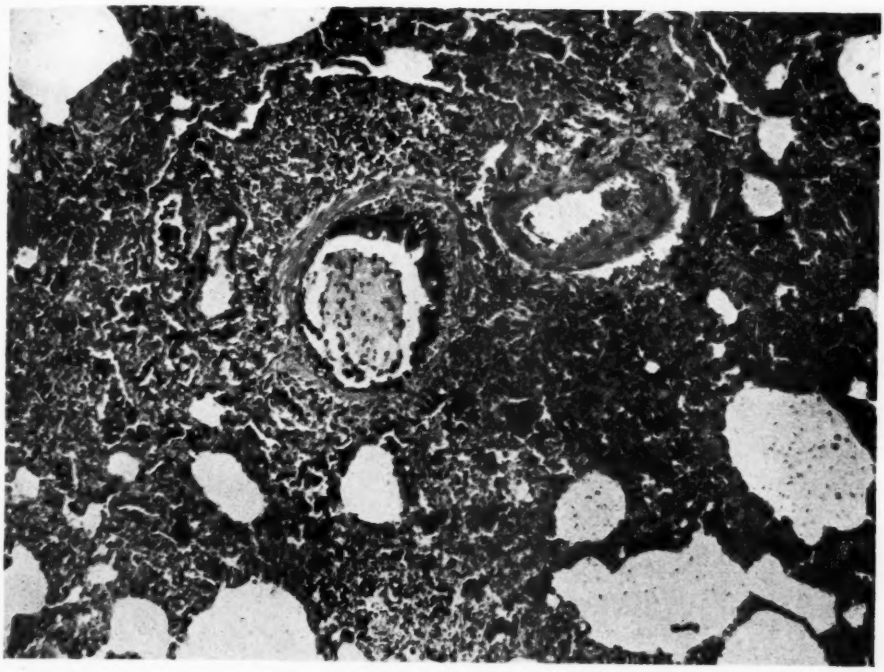

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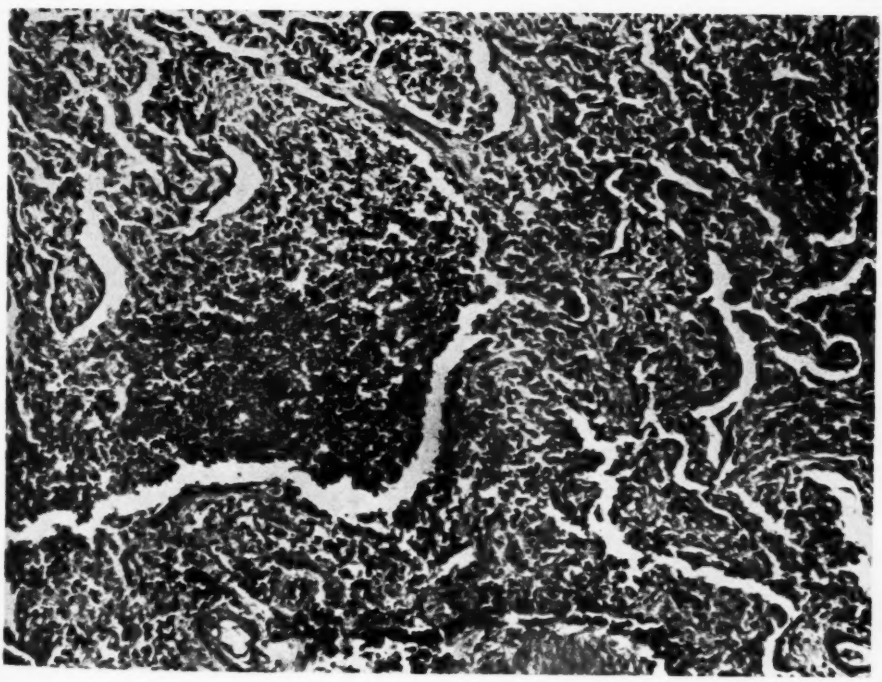

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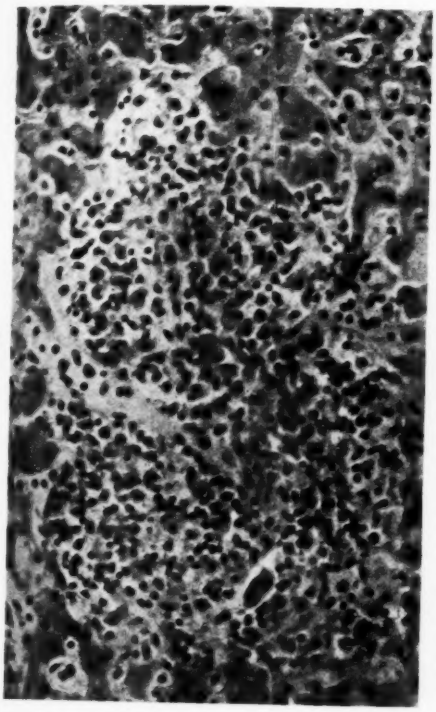
Plate 107

Fig. 37. Acc. 111021. Focal granulomatous inflammation near the midzone of a lobule of liver in a case of scrub typhus. Hematoxylin and eosin stain. × 230. Neg. 83011.

Fig. 38. Acc. 111021. Focal inflammation of liver in scrub typhus. Hepatic cells in center of focus have been lysed. Hematoxylin and eosin stain. × 450. Neg. 83012.

Fig. 39. Acc. 112241. Focal, midzonal granulomatous inflammation of liver in case of scrub typhus, showing evidence of phagocytosis by Kupffer cells. Hematoxylin and eosin stain. × 230. Neg. 83014.

Fig. 40. Acc. 105720-3A. Sickling of red blood cells within tributary of portal vein in liver from a case of epidemic typhus in an Egyptian. Hematoxylin and eosin stain. × 160. Neg. 83193.



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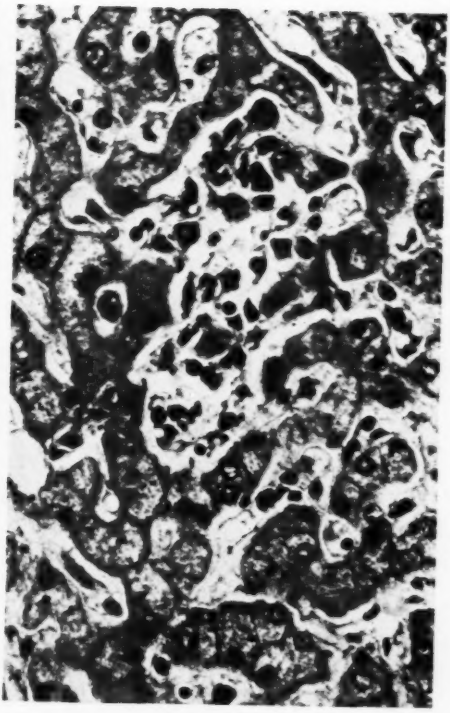

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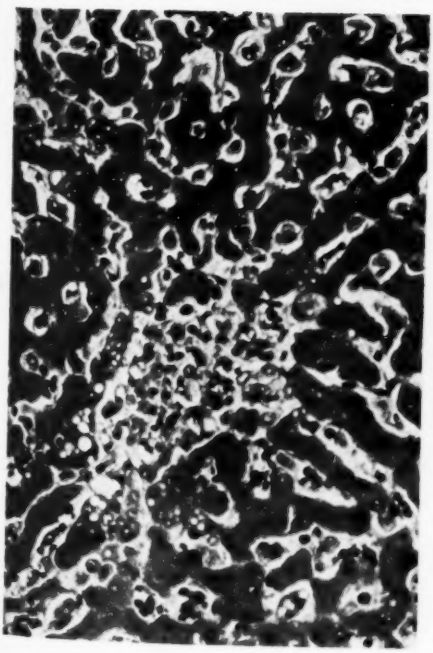

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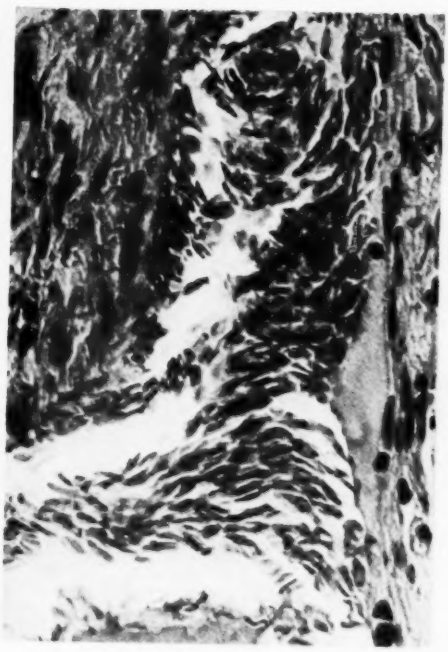

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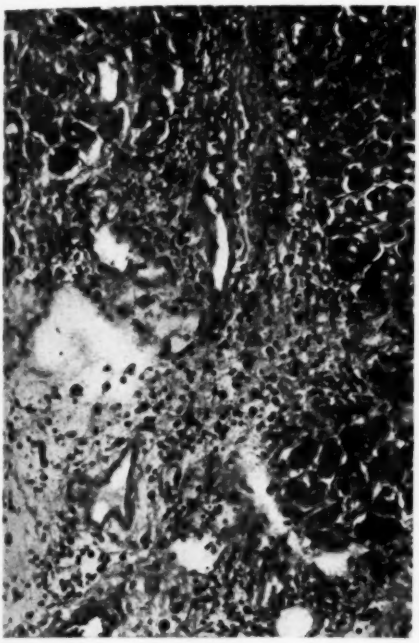
PLATE 108

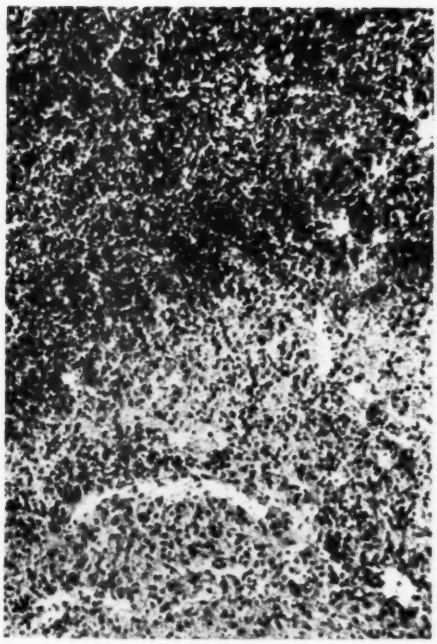
FIG. 41. Acc. 112449. Interstitial pancreatitis in scrub typhus. The infiltrate, as in other organs, consists of various mononuclear cells. Hematoxylin and eosin stain. × 145. Neg. 82894.

FIG. 42. Acc. 105720-16A. Necrosis of spleen in epidemic typhus. An identical change occurs in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82926.

FIG. 43. Acc. 94653. Section of spleen from a case of scrub typhus illustrating the large basophilic macrophage ("acute splenic tumor cell") characteristic of the infiltrate in all organs. Hematoxylin and eosin stain. × 515. Neg. 77035.

FIG. 44. Acc. 111024. Smear of spleen from case of scrub typhus showing rickettsiae in the cytoplasm of large mononuclear cells. Giemsa's stain, Wolbach's modification. × 1360. Neg. 83449.





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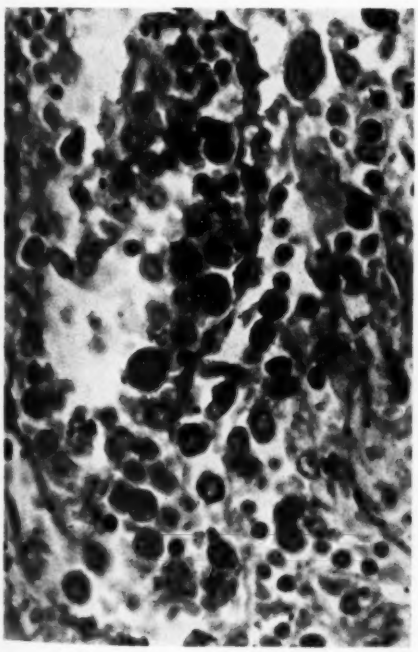

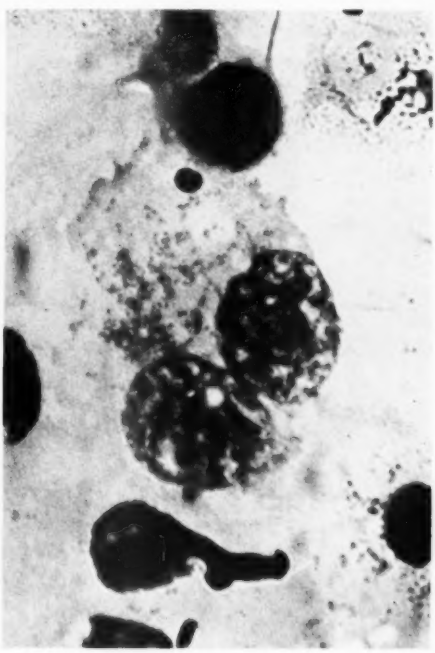

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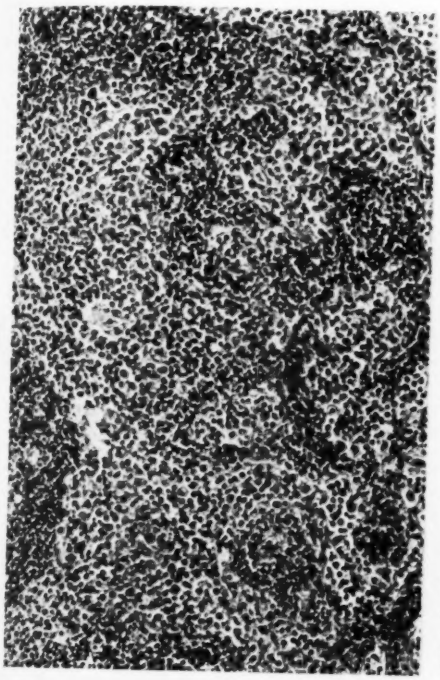
PLATE 109

FIG. 45. Acc. 112247. Lymph node from a case of scrub typhus showing dilatation of sinuses, principally with acidophilic macrophages, many of which are vacuolated ("blister" histiocytes) and contain cellular débris. "Sinus catarrh." Hematoxylin and eosin stain. × 120. Neg. 83145.

FIG. 46. Acc. 111023. Lymph node from a case of scrub typhus showing early necrobiotic changes beginning in a germinal center. Hematoxylin and eosin stain. × 145. Neg. 82890.

FIG. 47. Acc. 111023. Lymph node of scrub typhus with more advanced necrobiosis than that shown in Figure 46. Hematoxylin and eosin stain. × 120. Neg. 83901.

FIG. 48. Acc. 101690. Lymph node of scrub typhus showing extension of the inflammation through the capsule. Hematoxylin and eosin stain. × 230. Neg. 78155.



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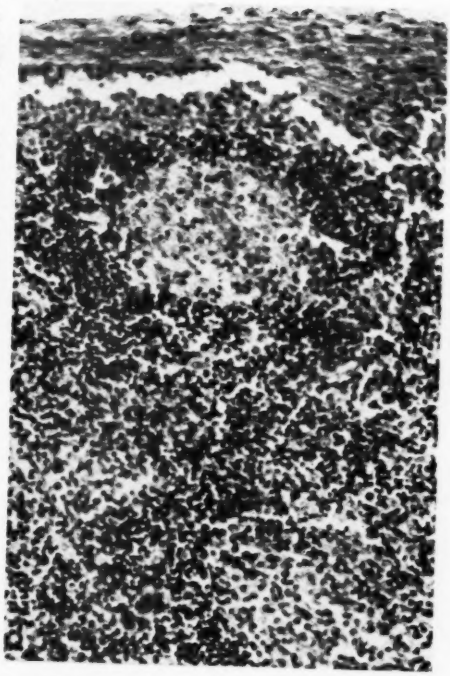

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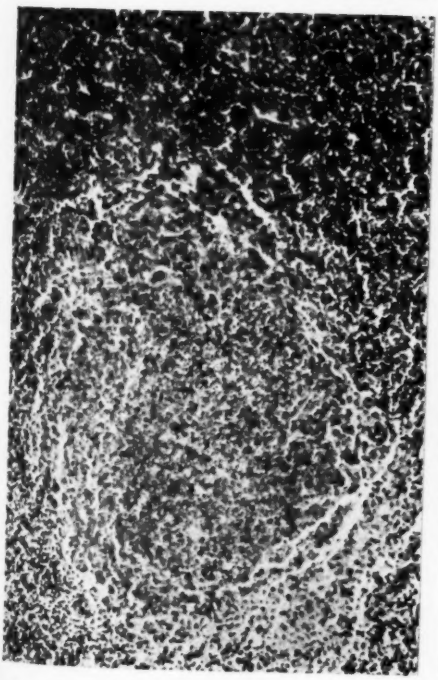

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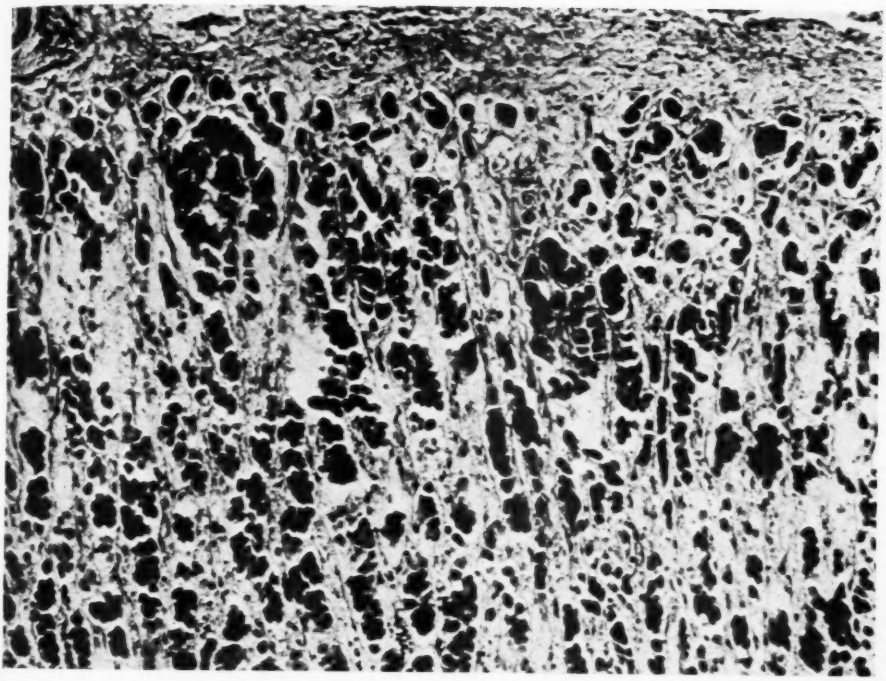
PLATE 110

FIG. 49. Acc. 112232. Adrenal gland from a case of scrub typhus showing marked "tubular degeneration" (Rich) of cortex. Hematoxylin and eosin stain. × 175. Neg. 82923.

FIG. 50. Acc. 112449. Adrenal gland from a case of scrub typhus showing abundant fat in midfascicular zone, rather than depletion. Hematoxylin and eosin stain. × 120. Neg. 83142.

FIG. 51. Acc. 105720-22A. Thrombo-arteritis of adventitial arteries of adrenal in a case of epidemic typhus. Hematoxylin and eosin stain. × 145. Neg. 80315.



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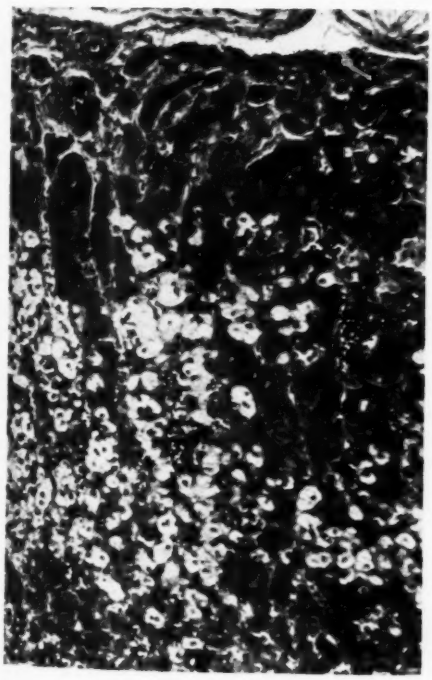

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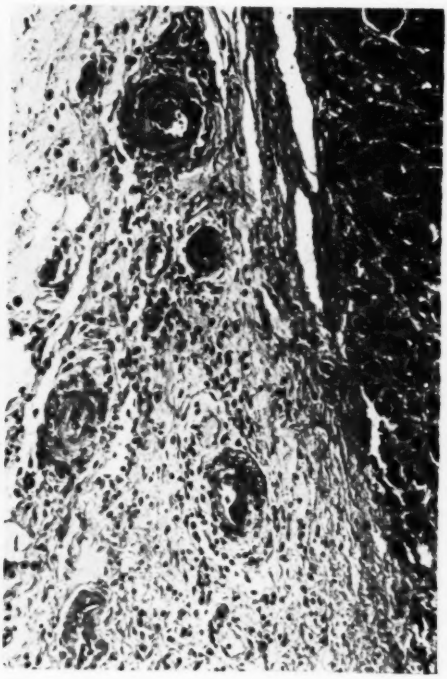

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Comparative Study of Scrub Typhus

PLATE 111

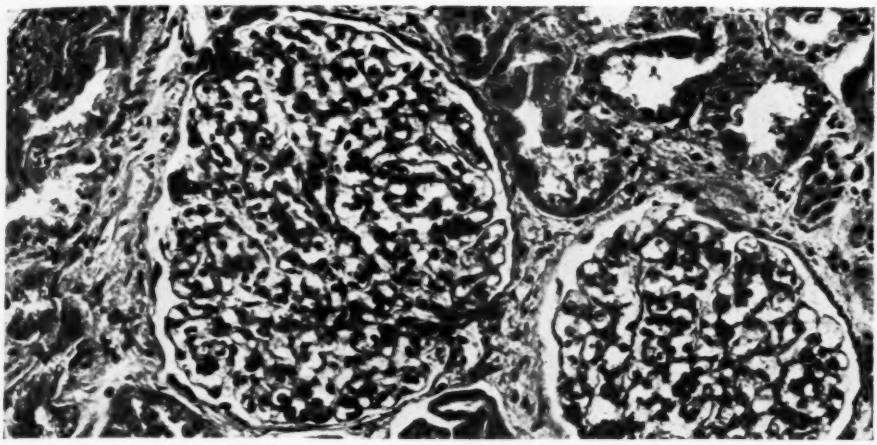
FIG. 52. Acc. 106767. Acute diffuse glomerulonephritis in a case of scrub typhus, showing particularly the absolute ischemia, the endothelial hyperplasia, and the fusion of capillary loops. Hematoxylin and eosin stain. × 255. Neg. 82917.

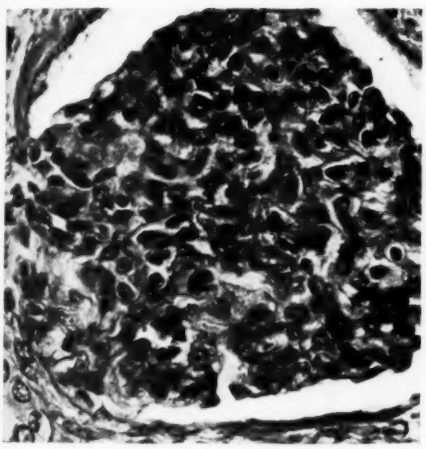
FIG. 53. Acc. 115681. Acute diffuse glomerulonephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 500. Neg. 82555.

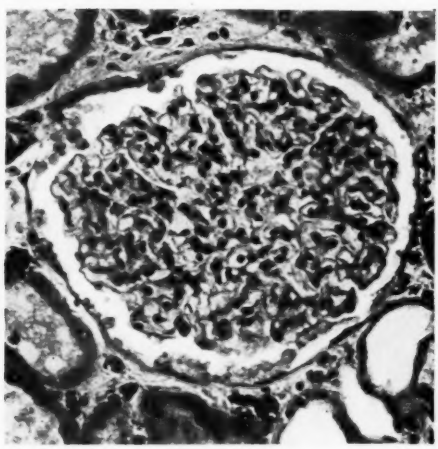
FIG. 54. Acc. 115682. Acute diffuse glomerulonephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 255. Neg. 82903.

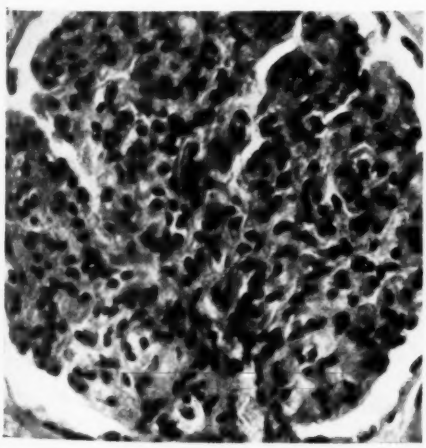
FIG. 55. Acc. 105720-20A. Acute diffuse glomerulonephritis in a case of epidemic typhus. Hematoxylin and eosin stain. × 350. Neg. 82559.

FIG. 56. Acc. 75608. Acute diffuse glomerulonephritis in a case of Rocky Mountain spotted fever. Hematoxylin and eosin stain. × 330. Neg. 83157.









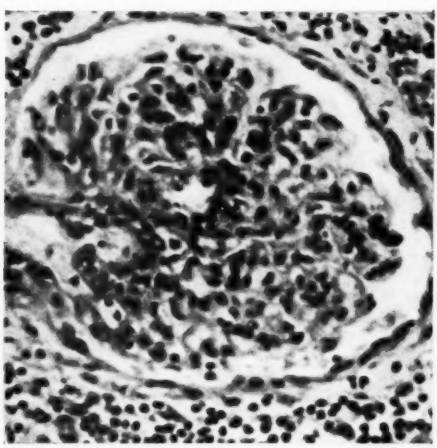


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PLATE 112

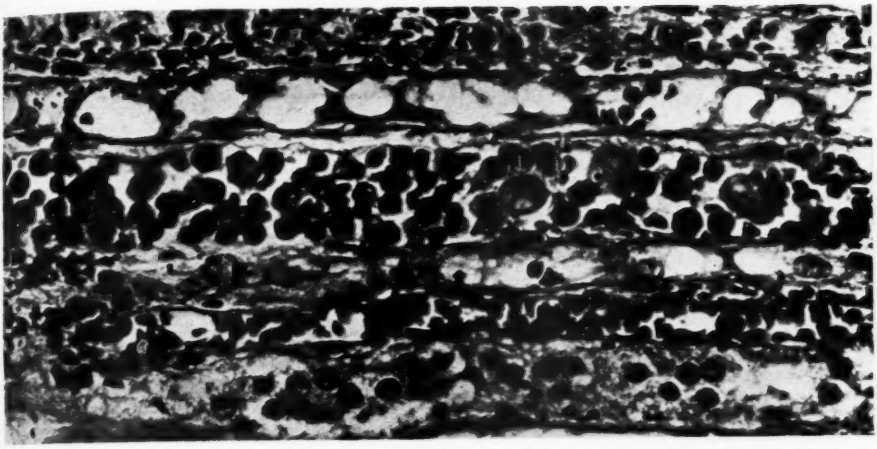
FIG. 57. Acc. 94537. Section of medulla of kidney in a case of scrub typhus, showing a striking concentration of mononuclear cells within the lumen of a peritubular vein. The cells include lymphocytes, plasma cells, possibly Türk cells, basophilic and acidophilic macrophages, the latter manifesting evidence of cytophagocytosis. Hematoxylin and eosin stain. × 500. Neg. 82550.

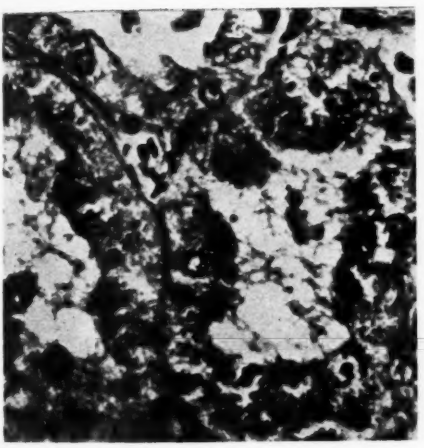
FIG. 58. Acc. 94655. Vacuolization and hyaline droplet formation within the epithelium of proximal convoluted tubules in a case of scrub typhus with no glomerulonephritis. Hematoxylin and eosin stain. × 500. Neg. 82558.

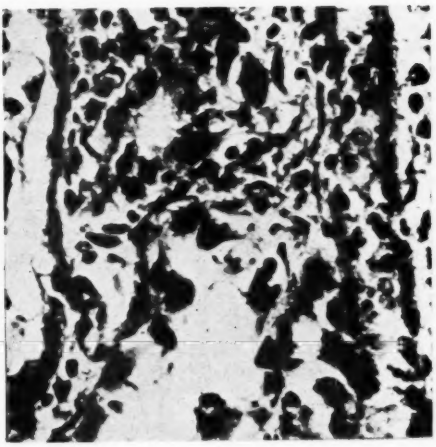
FIG. 59. Acc. 111605. Degeneration and regeneration of epithelium of distal tubules of glomerulonephritic kidney from a case of scrub typhus. A mitotic figure may be noted. Hematoxylin and eosin stain. × 500. Neg. 82560.

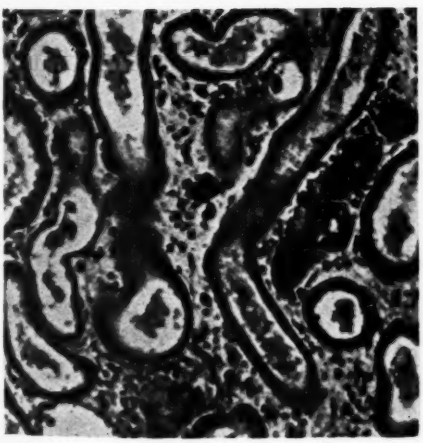
FIG. 60. Acc. 112262. Acute diffuse interstitial nephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82554.

FIG. 61. Acc. 111605. Phlebitis of interlobar vein in association with focal interstitial nephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 120. Neg. 82551.









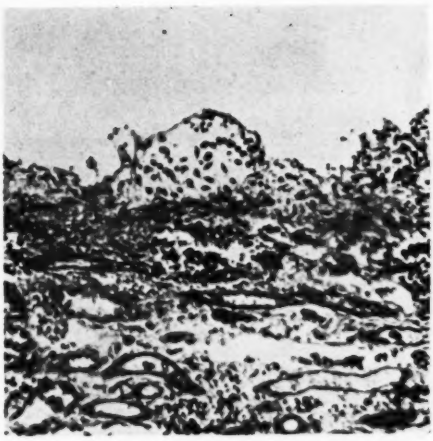


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PLATE 113

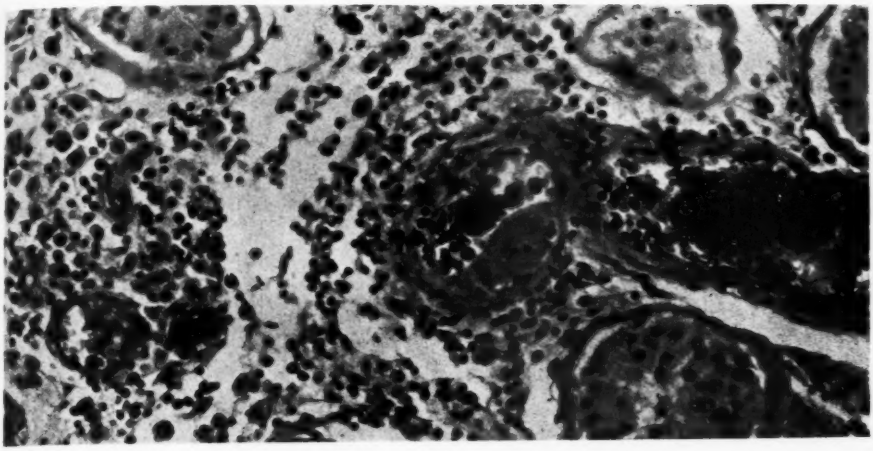
FIG. 62. Acc. 112247. Interstitial orchitis and thrombophlebitis in a case of scrub typhus. Hematoxylin and eosin stain. × 230. Neg. 82919.

FIG. 63. Acc. 112449. Tubular atrophy, and interstitial edema and inflammation in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82915.

FIG. 64. Acc. 112240. Fibrinoid degeneration of a segment of a testicular vein in a case of scrub typhus. There is evidence of atrophy even in the absence of appreciable interstitial inflammation. Hematoxylin and eosin stain. × 230. Neg. 82717.

FIG. 65. Acc. 105720-16A. Thrombo-arteritis in rete testis in a case of epidemic typhus. Hematoxylin and eosin stain. × 145. Neg. 82909.

FIG. 66. Acc. 105720-21A. Thrombo-arteritis with arterionecrosis, and periarterial mononuclear response in the tongue in a case of epidemic typhus. Hematoxylin and eosin stain. × 330. Neg. 80317.



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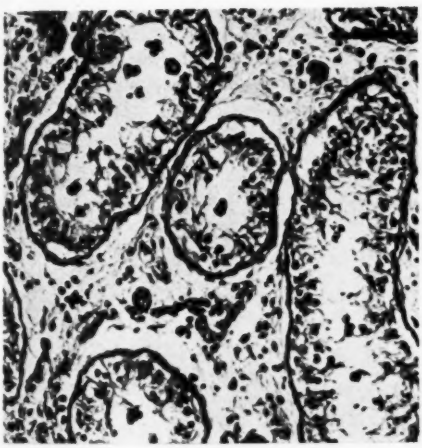

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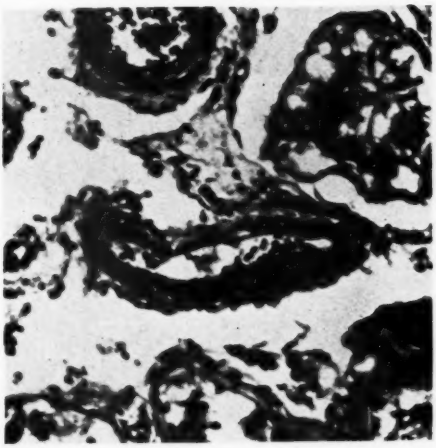

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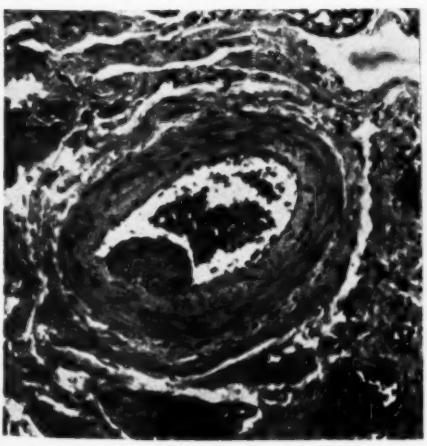

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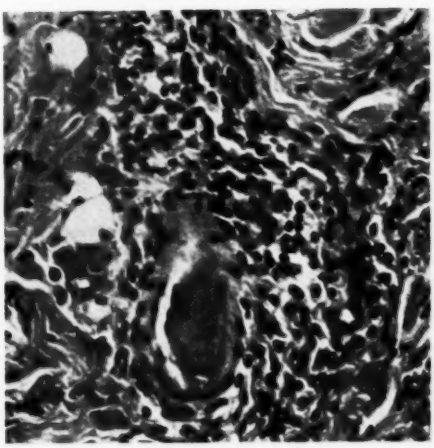

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PLATE 114

FIG. 67. Acc. 94537. Nodule in brain from a case of scrub typhus. Hematoxylin and eosin stain. × 450. Neg. 82939.

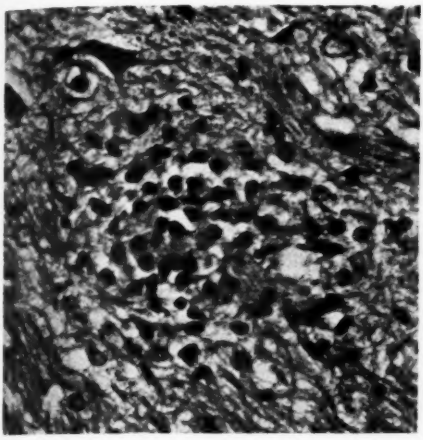
FIG. 68. Acc. 105720-13A. Nodule in brain from a case of epidemic typhus, under same magnification as Figure 67. The larger size and somewhat greater diversity of types of cells may be noted. Hematoxylin and eosin stain. × 450. Neg. 82929.

FIG. 69. Acc. 111023. Nodule of brain in scrub typhus showing the disruption of the argyrophilic, capillary wall. Wilder's silver stain. × 810. Neg. 82504.

FIG. 70. Acc. 105720-21A. Nodule of brain from a case of epidemic typhus showing the argyrophilic capillary wall. Wilder's stain. × 810. Neg. 82506.

FIG. 71. Acc. 79689. "Nodule" of brain from a case of Rocky Mountain spotted fever, showing a collection of cells about a prominent thrombosed arteriole. Hematoxylin and eosin stain. × 400. Neg. 82899.

FIG. 72. Acc. 79689. Early microinfarct of brain in Rocky Mountain spotted fever, with a central thrombosed arteriole and characteristically disrupted parenchyma. Hematoxylin and eosin stain. × 230. Neg. 83007.



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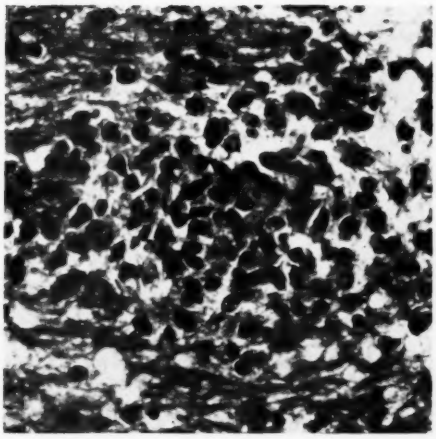

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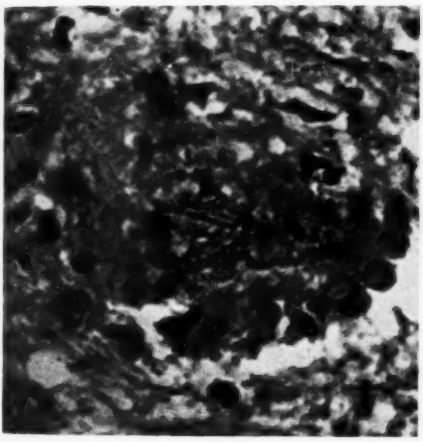

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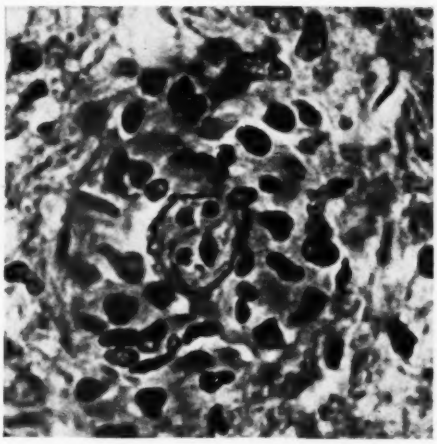

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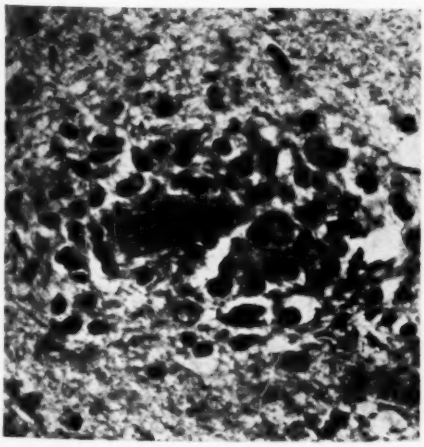

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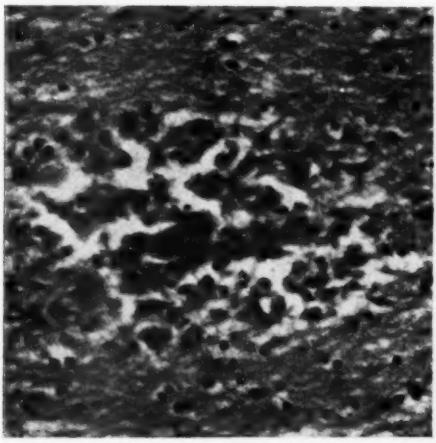

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Fig. 73. Acc. 112435. Marked leptomeningeal reaction in a case of scrub typhus. Hematoxylin and eosin stain. × 110. Neg. 83156.

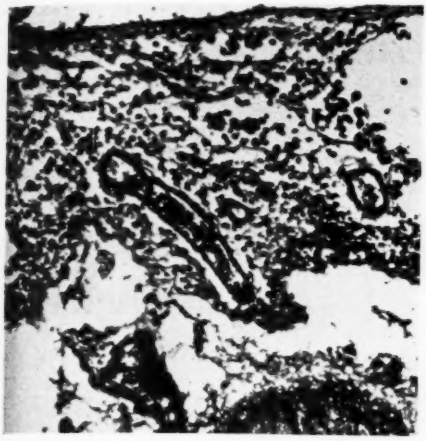
Fig. 74. Acc. 112435. Focal hemorrhages in the supra-optic nucleus in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82930.

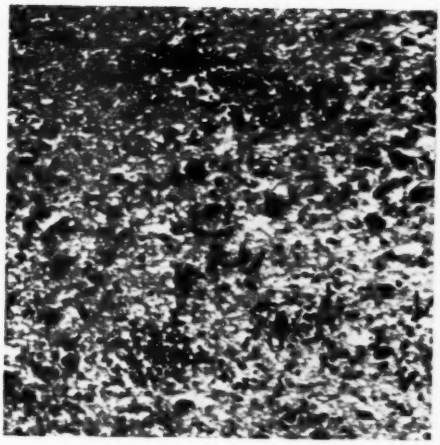
Fig. 75. Acc. 111020. Satellitosis, in cerebral cortex of a case of scrub typhus. Hematoxylin and eosin stain. × 500. Neg. 83005.

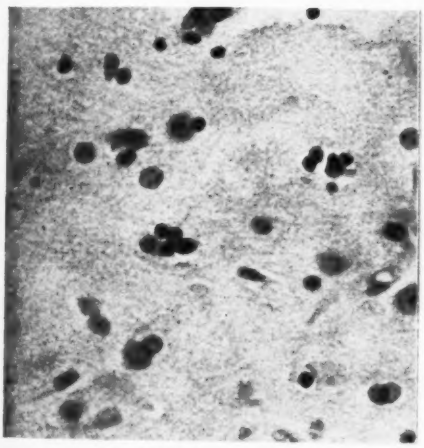
Fig. 76. Acc. 105720-22A. Thrombo-arteriolitis and perivascular mononuclear cell infiltration of posterior lobe of pituitary gland in a case of epidemic typhus. Hematoxylin and eosin stain. × 230. Neg. 82925.

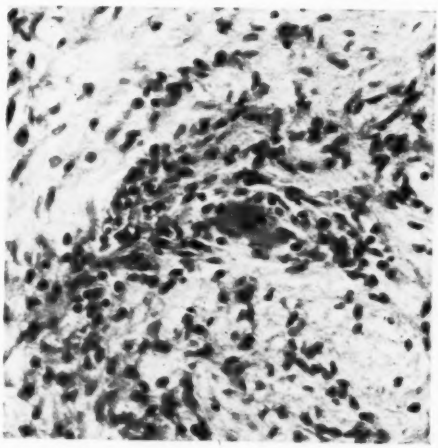
Fig. 77. Acc. 101508. Malarial "granuloma." There is a rosette of glial cells and a rim of demyelinization. Hemorrhage is absent in this instance. The parasites are visible in the capillaries as black dots. The malarial lesions tend to stimulate the microinfarcts of Rocky Mountain spotted fever more than they do the nodules of scrub or epidemic typhus. Hematoxylin and eosin stain. × 280. Neg. 77498.

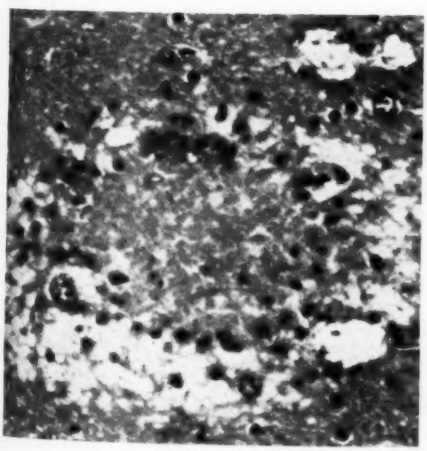
Fig. 78. Acc. 41410. A "nodule" in the brain from a case of Chagas' disease. This lesion tends to follow the pattern of scrub typhus or epidemic typhus. Hematoxylin and eosin stain. × 450. Neg. 83009.

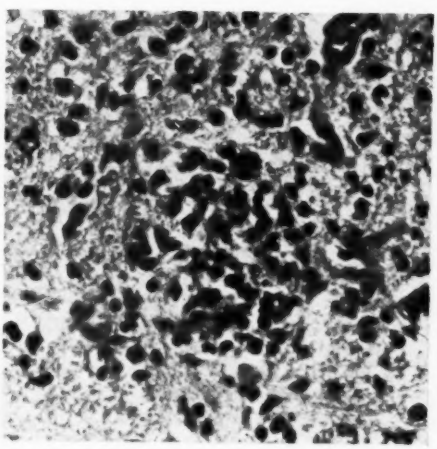












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Comparative Study of Scrub Typhus

PLATE 116

FIG. 79. Acc. 111026. A section of testis from a case of scrub typhus showing a mononuclear cell infiltration of the wall of an artery as if by extension from the interstitial infiltrate. Hematoxylin and eosin stain. × 145. Neg. 82893.

FIG. 80. Acc. 112247. Thrombophlebitis within the testis in a case of scrub typhus. The accompanying artery is spared. Hematoxylin and eosin stain. × 145. Neg. 82891.

FIG. 81. Acc. 94722. Pulmonary artery from a case of scrub typhus showing the type of subendothelial vacuolization and intimal edema that is associated with allergic manifestations, as in bronchial asthma. Interstitial pneumonitis was present in this instance. Hematoxylin and eosin stain. × 145. Neg. 83194.

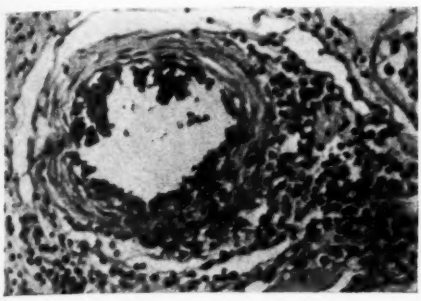
FIG. 82. Acc. 105720-18A. A cutaneous artery from a case of epidemic typhus showing either a thrombo-arteritis or a true, intimal verruca. The uniform granularity of the hillock and the underlying arterial wall, and the lifting of the endothelium are some of the reasons for considering the vasculogenesis of the intimal mound. The degeneration involves the media and adventitia as well as intima. Hematoxylin and eosin stain. × 230. Neg. 82896.

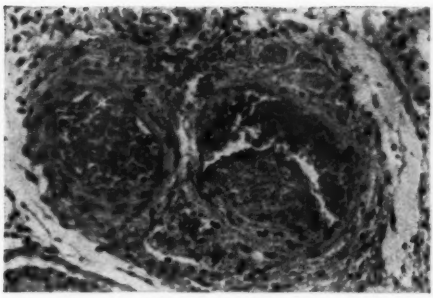
FIG. 83. Acc. 105720-10A. Acute diffuse glomerulonephritis in a case of epidemic typhus. This section illustrates another site of vascular lesions. There are swelling, hyperplasia and hyperchromasia of the endothelial cells of the glomerular capillaries as well as focal karyorrhexis and fibrinoid degeneration. Hematoxylin and eosin stain. × 255. Neg. 82902.

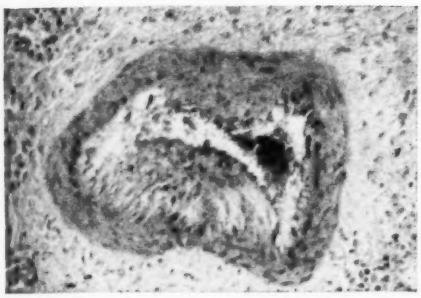
FIG. 84. Acc. 105720-20A. Sickling of red blood cells within an interlobular vein in a case of epidemic typhus in an Egyptian native. Hematoxylin and eosin stain. × 330. Neg. 83198.

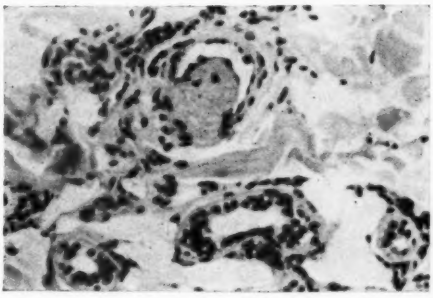
FIG. 85. Acc. 94875. Necrotizing arteritis in the midst of a focus of interstitial nephritis in a case of Rocky Mountain spotted fever. Hematoxylin and eosin stain. × 230. Neg. 82906.

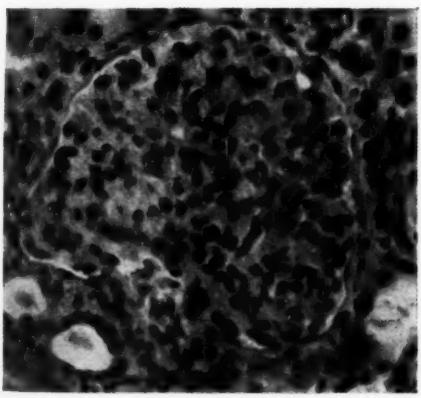
FIG. 86. Acc. 94423. Necrotizing arteritis of the scrotum from a case of Rocky Mountain spotted fever. ×255. Neg. 82903.

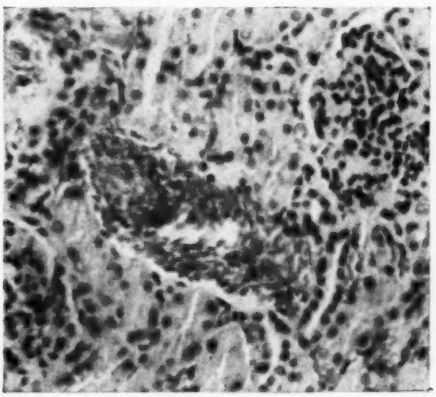


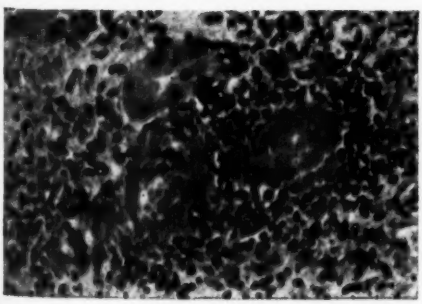


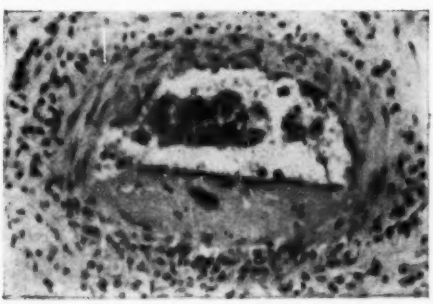












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Comparative Study of Scrub Typhus

MALIGNANT LYMPHOMA (SO-CALLED LEUKEMIA) IN DOGS *

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From the point of view of comparative pathology, the leukemic and leukemia-like diseases of the lower animals have engaged the attention of many investigators. Such studies have largely been directed toward a better understanding of similar diseases as they occur in man. The problem of leukemia in man finds its counterpart in the leukemia affecting animals.

With few exceptions, however, the disease in dogs has been only superficially examined, although it is fair to state that considerable information is at hand concerning the lymphomas as they affect this species. In many instances, hematologic and histologic studies were not performed and frequently the results of such examinations have been erroneously interpreted. The bewildering nomenclature of the human disease has often been applied to the canine disease without due consideration for the hematologic and pathologic alterations as they actually exist. While both the human and canine disease have many features in common, distinct differences occur and many of the classifications proposed for the former do not logically apply to the latter.

In this study, therefore, we propose to classify and describe the hematologic and pathologic aspects of the lymphomas in dogs, together with brief notes on the clinical features.

LITERATURE

The literature on the canine leukemias is very voluminous and many papers have been omitted from our bibliography due to lack of adequate pathologic and hematologic data.

Although Leisering [1] (1858) first described leukemia in the domesticated animals (in a horse), Siedamgrotzky [2] (1871) first reported lymphatic leukemia in a dog in which the lymph nodes and spleen were enlarged and the ratio of white to red cells was 1 to 15. Bollinger [3] (1874) designated as splenic and lymphatic leukemia a condition in a dog that showed hyperplasia of the spleen and lymph nodes, leukemic infiltrations of the liver and lungs, and a ratio of white to red cells after death of 1 to 5. Shortly after, Cadiot [4] (1892), Stockmann [5] (1893), Smythe [6] (1898), Olt [7] (1899), and others described cases of leukemia, principally of the lymphatic type, in dogs.

Weil and Clerc [8] (1904) were among the first to do blood examinations and noted lymphatic leukemia and aleukemic lymphatic leukemia

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in 2 dogs. In the first dog the white cells were 320,000: lymphocytes, 88 per cent; monocytes, 3 per cent; plasma cells, 1 per cent; polynuclear leukocytes, 8 per cent. The lymph nodes, liver, kidneys, spleen, mammary gland, and bone marrow showed lymphocytic infiltrations. The exact nature of the disease is unknown in the second case as these blood changes were absent and no autopsy was performed. In 1905, the same authors [9] reported 2 cases of myelogenous leukemia. A leukocytosis existed in both dogs: in one there were 165,000 white cells with 93 per cent polynuclear leukocytes and 7 per cent mononuclear leukocytes, and in the other the white cells totaled 50,000 with 88 per cent polynuclear leukocytes, 0.4 per cent eosinophils, 5.6 per cent monocytes, 4 per cent myeloblasts, and 2 per cent myelocytes. The bone marrow and other organs were thought to be infiltrated with myeloblasts. Opie [10] (1928) stated that the blood changes in these 2 cases do not establish the diagnosis of leukemia. Additional examples of leukemia in dogs, the majority of them being of the lymphogenous type, were noted by Cadiot and Weil [11] (1904), Lellmann [12] (1904), Jakob [13] (1907), Feuereissen [14] (1907), Jäger [15] (1907), and Ball [16] (1912). Lüdke [17] (1910) designated a condition in a dog as myelogenous leukemia from the blood and tissue changes. According to Wirth [18] (1931), the blood findings in the cases of Olt,[7] Jakob,[13] and Abendroth [19] (1913) could be termed myelogenous and considered as an expression of myelosis. Dahlström and Henschen [20] (1918) collected 50 cases of leukemia in dogs from the literature and concluded that the disease is not particularly frequent. Crocker [21] (1919) cited 1 case of leukemia from autopsy examinations of 1548 dogs. Milks [22] (1919) noted pseudoleukemia in a dog in which the leukocytes were normal, the spleen and lymph nodes were greatly enlarged, and these organs and the liver were infiltrated with lymphocytes. Spaulding [23] (1920) mentioned 6 cases of leukemia and 7 of pseudoleukemia in a group of about 15,000 dogs. In 1 animal the white cells were increased and the differential was: polynuclear leukocytes, 49 per cent; mononuclear leukocytes, 11 per cent; lymphocytes, 3 per cent; and eosinophils, 37 per cent. Burnett [24] (1920) properly criticized the criteria by which the diagnosis of leukemia was made by Spaulding since hematologic data were not characteristic. Burnett further stated that myelogenous leukemia has not been reported in the domesticated animals.

Wirth [25] (1920) described 2 cases of lymphatic leukemia and 11 of myelogenous leukemia in dogs. In the former, in 1 animal the leukocytes were 24,000 with 60.1 per cent polynuclear leukocytes and 39.9 per cent lymphocytes; and in the latter the white cells were twice the normal number, with 43.2 per cent lymphocytes. Opie [10] considered

these cases as examples of aleukemic lymphoma rather than leukemia. In the 11 dogs, Wirth stated that although the typical blood pictures of myelogenous leukemia as they occur in man were absent, the histologic data were suggestive of myelogenous leukemia or subleukemia, as judged by the myeloid cells in the bone marrow and other organs. Lund [26] (1927) found 2 cases of myelosis in 6 dogs and Julliard [27] (1928) believed that myeloid leukemia is predominant in this species. Opie [10] critically reviewed the literature concerning leukemia in animals and concluded that in dogs, lymphatic leukemia occasionally occurs and that lymphomatous tumors are not uncommon. The nature of the disease described as myelogenous leukemia is as yet uncertain.

Knuth,[28] (1929) in his review of animal leukemias, quoted Weil and Clerc [9] and Wirth [25] to indicate that although the microscopic immature myeloid infiltrations are typical of myelosis, the blood picture is that of a leukocytosis. Milks and Olafson [29] (1929) described pseudoleukemia in a dog with 12,000 white cells and 77 per cent polynuclear leukocytes, 19 per cent monocytes, and 4 per cent lymphocytes. The lymph nodes, spleen, liver, and pancreas were enlarged and infiltrated with lymphocytic cells. Fourie and Ziehn [30] (1930) reported lymphoid aleukemia in a dog; *viz.*, a generalized lymphosarcomatosis, with 3000 white cells and a differential of 77 per cent lymphocytes, 23 per cent polynuclear leukocytes and monocytes. Wirth [18] (1931) stated that in the dog the myelosis constitutes approximately 80 per cent and the lymphadenosis comprises between 10 and 20 per cent.

Concerning the etiologic factors, Share-Jones [31] (1927) and Jármai [32] (1933) observed the apparent development of lymphogenous leukemia in connection with trauma. The history sometimes suggested that the disease was brought about by an intercurrent condition such as pregnancy (Stockmann [5]), overexertion (Timm,[33] 1919), 5 days after mating (Smythe [6]), and dochmiasis (Henschen [34]).

Feldman [35] (1932) classified leukemia, pseudoleukemia, etc. as lymphoblastoma with lymphoma as the benign and lymphocytoma as the malignant type. He suggested the use of leukemic or aleukemic before lymphocytoma to designate the hematologic changes. Myelogenous leukemia was termed myeloblastoma and he referred to the studies of Weil and Clerc [9] and Wirth [25] as contributing to the knowledge of the hematology associated with this condition. Wirth and Baumann [36] (1933) reported lymphatic leukemia with typical blood pictures and further stated that all cases in dogs are lymphatic although the blood picture may be that of a leukocytosis, thus reversing Wirth's [18, 25] previous beliefs. Hutyra, Marek, and Manninger [37] (1938) described the leukemias in animals, the descriptions being largely based

on the studies of some of the previously mentioned investigators. Collins [38] (1939) reported an instance of leukemia with normal white cell and differential counts. The lymph nodes were enlarged and the kidneys, spleen, and liver grossly normal. Cherry [39] (1940) noted lymphatic leukemia with 26,050 white cells and macroscopic lymphadenopathy and normal spleen and liver. Atkinson [40] (1941) described a case of leukemia with 56,040 leukocytes and a differential count of 45 per cent segmented neutrophils, 6 per cent eosinophils, 43 per cent lymphocytes, and 6 per cent monocytes. The liver, spleen, and lymph nodes were enlarged. In the latter 3 cases, no microscopic reports were given and the diagnosis of leukemia is questionable. Probably the most accurate statement pertaining to the leukemias in dogs was that of Engelbreth-Holm [41] (1942), who considered that lymphogenous leukemia has somewhat peculiar features and that two different types of the disease occur: one form corresponds to that observed in other animals and in man; the other has the same typical visceral lesions but shows changes in the blood that are not typical of lymphogenous leukemia but rather resemble nonspecific leukocytosis. The latter form constitutes the majority of cases in this animal.

Material and Methods

The cases studied represent dogs brought to the small animal hospital of one of us (F. B.) for treatment. Blood examinations were performed by the usual methods. Bone marrow was aspirated from the crest of the ilium and the percentage distribution of marrow cells determined by the previously described morphologic criteria.[42, 43] Dry imprint preparations of lymph nodes secured for biopsy, and of lymph nodes and spleen made immediately after killing the animal with soluble pentobarbitol were stained with Wright's and May-Grünwald-Giemsa stains. In some instances, Isaac's method [44] (brilliant cresyl blue and Wright's) was employed to stain the blood, aspirated marrow, lymph node imprints, and smears of lymph nodes previously macerated in the serum of the same animal. Necropsies were performed immediately and the tissues were fixed in Zenker's formalin solution. The sections were stained with hematoxylin and eosin, Dominici's stain,[45] and Wilder's reticulum stain.[46]

Classification

A variety of terms such as lymphoblastoma, lymphoma, lymphosarcoma, leukemia, pseudoleukemia, lymphadenosis, lymphocytoma, lymphomatosis, and others have been applied to tumor-like enlarge-

ments of the lymph nodes in both human and veterinary medicine. We have followed the terminology of Gall and Mallory [47] in designating the diseases we encountered in dogs as malignant lymphoma since in our opinion it best expresses the anatomic-pathologic features present.

A study of the imprint and sectioned lymph nodes revealed four distinct categories into which the cases could be subdivided on the basis of the cytologic structure of the predominant cell type. These were as follows: (1) lymphoblastic type (6 cases); (2) lymphosarcoma cell type (1 case); (3) mixed cell type (4 cases); (4) lymphocytic type (2 cases). Stem cell and clasmatocytic lymphomas,[47] Hodgkin's disease, and follicular lymphoblastoma were not encountered.

In all forms there occurred in the imprint material occasional polymorphonuclear leukocytes, eosinophils, tissue mast cells and pathologic lymphocytes, histiocytes often with phagocytosed material, cells in mitoses, and lymphocytic cells other than those of the principal cell type. It must be emphasized that the cellular type peculiar to each case was universally found in all tissues and organs showing lymphomatous infiltrations. In addition, in those animals in which biopsies of lymph nodes were done, there was no evidence of cellular dedifferentiation as noted by Gall and Mallory [47] or of cellular differentiation as observed by Ehrlich and Gerber.[48]

1. *Lymphoblastic Type.* In imprints the cells of lymphoblastic type were round or oval, occasionally irregular, and varied from 9.12 to 18.42 μ with an average diameter of 13.68 μ (Fig. 1). The round or oval nucleus had a distinct membrane and ranged from 8.4 to 14.53 μ with an average of 11.18 μ. It was usually eccentrically located in the cytoplasm. The nuclear structure was leptochromatic with a very fine, regular chromatin network that presented a sieve-like appearance. The parachromatin was abundant and consisted of numerous minute, spherical granules imbedded in the chromatin. There were from 1 to 4 nucleoli, which in 2 cases were single and large with only an occasional cell containing multiple nucleoli. In the remaining 4 cases the majority of cells had smaller multiple nucleoli. In the former type the nucleoli were either central or peripheral, while in the latter they were irregularly scattered in the nucleoplasm. They were round or oval and the chromatin was condensed at their margins. The cytoplasm was moderately basophilic and Auer bodies and azure granules were absent. The blue-staining spongioplasm consisted of small, granular masses in the lighter hyaloplasm so that the cytoplasm appeared somewhat foamy, mottled, and vacuolated. The basophilic spongioplasm was usually more abun-

dant in the periphery of the cell so that it appeared darker, condensed, and homogeneous. A few small cytoplasmic vacuoles occurred in some cells and the perinuclear zone was often a lighter blue.

In sections the lymphoblasts varied from 4.93 to 11.1 μ and averaged 8.02 μ in diameter (Figs. 5 and 6). They were round or oval when loosely arranged and were often polyhedral and irregular when densely massed. The nuclei were round or oval, occasionally irregular, measured from 4.46 to 8.5 μ with an average of 6.39 μ, and were usually eccentrically located. The nucleus was vesicular in appearance with relatively few fine chromatin granules that were frequently marginated on the deeply staining nuclear membrane. All cells contained from 1 to 4 round or oval, prominent acidophilic nucleoli that often had accumulations of chromatin granules at their peripheries. The relative size and the distribution of the nucleoli were similar to those in the imprint material. The cytoplasm was homogeneous, vacuoles were occasionally present, and granules were absent. With hematoxylin and eosin the cytoplasm was amphophilic and with the Dominici stain it was blue.

2. *Lymphosarcoma Cell Type.* Imprints of cells of lymphosarcoma type stained with brilliant cresyl blue and Wright's stain revealed cellular characteristics that easily differentiated them from other cell types (Fig. 2). These cytologic features were less discernible with the May-Grünwald-Giemsa's stain or with Wright's stain alone. The round or oval cells measured from 8.78 to 14.4 μ with an average of 11.18 μ. The cytoplasm was moderately basophilic and occasionally contained small vacuoles. Azure granules and Auer bodies were absent and the perinuclear cytoplasm was often a lighter blue. The nuclei ranged from 6.73 to 12.0 μ with an average of 9.7 μ and were round, oval, or slightly indented. Two distinct types of nuclear structure existed with definite transitional stages between them. The nucleus of the immature lymphosarcoma cell was larger and had a leptochromatic structure similar to that of the lymphoblast. Other nuclei were more mature with heavy blocks of deeply staining chromatin of a pachychromatic appearance. Numerous transitional stages were observed between these two cell types and the majority of cells were of these intermediate forms. Each nucleus contained from 1 to 6 nucleoli that showed characteristic features with the brilliant cresyl blue and Wright combination. They stained a deep blue and were intimately surrounded by a narrow zone of deep purplish blue perinucleolar chromatin material. Application of the same staining procedure to cells of the other types indicated that the nucleoli did not stain in the same distinctive fashion and that nucleoli were absent in the lymphocytic type.

In sections the cells measured from 4.89 to 8.7 μ with an average of 6.56 μ and the nuclei from 4.63 to 6.51 μ with an average of 5.57 μ. In all detailed morphologic aspects, however, the lymphosarcoma cells appeared identical with the lymphoblasts (Fig. 7).

3. Mixed Cell Type. The imprints from cases with cells of mixed type revealed three distinct cell types that occurred in approximately equal proportions, although in some fields one or another cell form sometimes predominated (Fig. 3). These consisted of large lymphoblasts identical with those in the lymphoblastic type, small mature lymphocytes, and cells intermediate between these. The last were larger than the mature lymphocytes but were usually smaller than the lymphocytes. Their nuclear structure was immature but evidenced early pachychromatism and small nucleoli were only occasionally present. Measurements of approximately equal numbers of the three cell types indicated a variation of 6.4 to 17.2 μ with an average of 11.3 μ. The nuclei ranged from 6.35 to 13.7 μ and averaged 9.6 μ.

In sections the three cell types, namely, large lymphoblasts, mature lymphocytes, and transitional forms, were likewise present (Fig. 8). They varied from 4.59 to 14.66 μ with an average of 7.44 μ and the nuclei ranged from 4.0 to 10.78 μ with an average of 6.13 μ.

4. Lymphocytic Type. In imprints the cells of lymphocytic type measured from 4.97 to 10.15 μ with an average of 8.41 μ and the nuclei from 4.68 to 10.0 μ with an average of 7.55 μ. They had the morphologic appearances of mature lymphocytes as seen in the peripheral blood (Fig. 4). The nucleus was round or oval, occasionally indented, and nucleoli were absent. The chromatin was aggregated into coarse, block-like masses that contrasted with the parachromatin, producing a pachychromatic appearance. The nucleus was surrounded by a narrow zone of slightly basophilic cytoplasm that sometimes contained a few, small, azure granules.

In sections the cells were from 4.33 to 6.0 μ in diameter, with an average of 4.96 μ, and the nuclei varied from 4.0 to 4.88 μ with an average of 4.39 μ. Morphologically, the cells resembled the small mature lymphocytes as they appear in sectioned material (Fig. 9). The nuclear structure consisted of coarse, deeply staining, angular chromatin blocks; nucleoli were absent, and there was a thin rim of acidophilic cytoplasm.

Clinical Data

Incidence. In a group of 10,000 dogs, 20 cases of malignant lymphoma were encountered, representing an incidence of 0.2 per cent.

Age. The age varied from 5 to 12 years and averaged 9.08 years.

The disease thus occurs in older animals, as the normal dog usually lives from 10 to 14 years, barring death from accidents.

Sex. Of the 20 animals, 12 were males and 8 were females. This sex incidence differs from that found by other investigators, since in any larger group of cases females are rarely affected, as is true in man.

Breed. The breed incidence was as follows: Scottish Terriers, 7 cases; Boston Terriers, Cocker Spaniels, Chow Chows, 2 cases in each; Wire-haired Fox Terrier, German Shepherd, 1 case each; and mongrels, 5 cases. Although no definite conclusions can be derived from a small series, it appears significant that more Scottish Terriers were affected than the other breeds. This animal has become very popular in this country during the past 7 years but the increased numbers alone cannot be responsible for the increased frequency as other breeds, such as the Cocker Spaniel, occur in equal if not larger numbers.

Duration. It is obviously difficult, if not impossible, to determine the exact duration of the disease because of uncertainty as to its inception. In 8 cases a complete history was obtained from the owners, who stated that the animals were ill from 3 days to 5 months and averaged 43 days. Following the diagnosis, the dogs were hospitalized until death supervened, or was imminent so that the animal was killed. They survived from 13 to 64 days with an average of 38 days. In 5 dogs of this group the past history was known so that the total length of illness from its inception until death varied from 39 to 163 days and averaged 99.4 days. The course in our series corresponds with that reported by others although the disease when first recognized has in some instances lasted from 1 to 3 years.[7, 18]

Symptoms. In the early stages of the disease, the animals were apparently normal with the exception of "lumps" or swellings as reported by the owners. Later the dogs became less active and weaker, vomited occasionally, had diarrhea or were constipated, ate poorly; some showed thirst and polyuria, others coughed and breathed heavily. The temperature was normal although occasional slight elevations occurred. Hemorrhages, as reported by others as being similar to those in man, were absent. In the terminal stages, the animals were thin, cachectic, had purulent ocular and nasal discharge, and pale mucous membranes. Bilateral exophthalmus occurred in 2 cases, jaundice in 1, ascites in 2, and proptosed nictitating membranes in 4.

Observations upon the Blood and Bone Marrow

Table I summarizes the results of the blood and marrow examinations and the average normals [42] are included as a basis for comparison. Additional blood studies were made on 3 animals that were not autopsied and these showed changes similar to those listed in the table.

Peripheral Blood. In the early stages the red cells and hemoglobin of the peripheral blood were within the normal range. As the disease progressed, the erythrocytes and hemoglobin decreased so that a definite anemia existed in the terminal stages. Normoblasts sometimes occurred in considerable numbers and the red cells showed anisocytosis, polychromatophilia, poikilocytosis, and shadow forms.

In all animals except no. 4538 in which there was a leukopenia, the white cells were increased, sometimes to a considerable extent. This elevation resulted from greater numbers of nonsegmented and segmented neutrophils in most instances. Toxic changes were infrequent. With the exception of no. 7000, the relative number of mature lymphocytes was in the normal range or decreased. Lymphoblasts appeared terminally in large numbers in 1 animal only (no. 8847). Pathologic lymphocytes, identified by a pachychromatic nucleus without nucleoli, a deeply basophilic cytoplasm, and vacuoles in the cytoplasm and sometimes in the nucleus, occurred in 12 dogs. These cells, however, are not specific for the lymphomas as they are frequently present in the blood of normal dogs and in those with a wide variety of diseases. The other cellular types showed no noteworthy alterations and the blood platelets appeared normal. Changes of the blood suggestive of acute or chronic lymphatic leukemia as seen in man were absent in our cases.

Bone Marrow Examined by Biopsy. Differential counts of the aspirated marrow revealed lymphomatous involvement in 7 cases, in contrast to 11 cases in which lymphomatous involvement was determined by a study of sections of the bone marrow. This discrepancy will be discussed later in presenting the microscopic findings in the sections. In general, the aspirated marrow showed hyperplasia of myeloid cells, consisting principally of nonsegmented and segmented neutrophils, with an increase in neutrophilic myelocytes in several animals. In most instances the erythroid cells were decreased with consequent increase of the myeloid-erythroid ratio. The other cell types showed no noteworthy alterations.

Macroscopic Observations

No marked differences were observed in the gross pathologic changes of the different cellular types, so that all forms will be described together.

Lymph Nodes. With the exception of dog 4538, the superficial nodes were increased in size and varied from 1.5 to 9.5 cm. in diameter. This enlargement was usually bilateral and involved the cervical, mandibular, prescapular, axillary, peripenile, inguinal, and popliteal nodes. The degree of enlargement varied in the different cases and in the different nodes of the same animal although the cervical and mandibular groups were usually more prominent. They were nonadherent to the

TABLE I

Blood and Bone Marrow of Necropsied Dogs with Malignant Lymphoma

Type	Normal	Lymphocytic		Mixed cell					Lymphosarcoma cell				
Case number		4538	7838	1474	2737	7000	7067		8318				
							1/26	2/24	9/17	9/25	10/1	10/9	10/13
Peripheral blood													
Red cells÷1000	6629	3370	4490	4020	3990	4080	7090	3670	3560	2760	3030	3530	3880
Hemoglobin (gm. per 100 cc.)	12.2	5.1	7.6	6.8	6.8	8.0	12.4	5.4	6.74	5.29	4.8	6.9	5.6
White cells÷100	135	25	768	836	515	199	586	330	214	190	216	204	460
Myelocytes		3.0					0.33						
Non-seg. neutrophils	5.3	65.0	12.2	18.0	25.4	7.0	27.99	29.5	14.0	14.5	12.5	24.0	36.0
Segmented neutrophils	67.0	14.0	66.0	39.5	56.4	39.0	66.66	50.0	68.0	74.5	61.0	66.5	43.5
Eosinophils	3.9		2.2		1.6			1.5		2.0			
Basophils	0.3											0.5	
Monocytes	2.8	1.5	11.2	6.0	2.6	2.5			1.5	2.0	4.0	2.0	2.0
Lymphoblasts											0.5	1.0	1.5
Lymphocytes	20.7	12.5	7.4	21.5	13.2	41.0	6.33	17.5	13.0	8.5	17.5	6.0	9.5
Pathologic lymphocytes	0	4.0	1.0	15.0	0.8	10.5	0.66	3.0	2.0	0.5	2.5	0.5	7.5
Normoblasts (no. per 100 white cells)	0.6	4.5	0.2	1.5	26	14.5			29.5	37.5	48.5	56.0	13.5
Bone marrow													
Cells per cc.÷1000	144				100				293	118	149	50	
Megakaryocytes per cc.	41.2				33				None	11	None	11	
Myeloblasts	0.58	3.4	0.6	0.2	0.2			2.0					0.4
Myelocyte neutrophils	3.76	17.2	10.2	7.2	2.2			22.6		1.6	0.4	0.4	2.4
Myelocyte eosinophils	0.26				0.2								
Non-seg. neutrophils	23.5	37.4	28.4	19.2	23.0			40.3	17.0	10.6	33.6	13.8	8.8
Non-seg. eosinophils	0.12	0.2									0.6		
Segmented neutrophils	18.5	0.6	24.0	3.8	38.6			8.3	4.2	10.8	7.8	37.2	5.6
Segmented eosinophils	1.56	0.4	0.4	0.6					0.8	0.4		0.2	
Segmented basophils	0.02												
Heterophils	0.02												
Megaloblasts	1.02	0.8	1.6	0.6				2.6		0.4			
Erythroblasts	2.5	1.2		1.2	0.8			0.3					
Normoblasts	35.18	20.6	10.8	11.4	24.6			11.6	62.0	13.8	21.4	8.8	2.8
Monocytes	1.2		0.4	0.2	1.4					1.6		0.4	0.8
Monoblasts	0.14												
Lymphocytes	9.8	12.6	22.2	54.0	8.4			12.0	4.0	18.8	17.0	27.4	20.0
Pathologic lymphocytes	0.04	1.8	0.2							0.8	0.2	1.6	2.0
Lymphoblasts		0.4	0.2	0.4					6.4	0.2	1.2		
Mature lymphosarcoma cell									2.2	29.6	9.6	7.8	46.8
Immature lymphosarcoma cell									2.8	8.8	7.4	1.8	9.2
Plasma cells	0.82	2.6	0.8	1.0	0.2					0.4			
Hematogones	0.44	0.4			0.4				0.4	3.0	0.8	0.6	1.2
Reticulo-endothelial cells	0.54	0.4	0.2	0.2					0.2				
Myeloid-erythroid ratio	1.36:1.00	2.61:1.00	5.90:1.00	1:33:1.00	2.52:1.00			5.04:1.00	0.35:1.00	1.64:1.00	1.98:1.00	5.86:1.00	6.14:1.00

TABLE I (*Continued*)

Blood and Bone Marrow of Necropsied Dogs with Malignant Lymphoma

Lymphoblastic																	
6021	5829	8656		7760		6088				8847							
		1/26	2/15	4/24	5/15	3/11	3/23	3/27	4/9	4/18	4/25	5/2	5/9	5/17	5/24	6/7	6/18
6230	4850	6700	4170	4440	4370	4150	4440	4480	2505	6880	5960	6520	5100	4060	4650	4680	3800
11.3	7.4	11.8	6.9	7.0	6.8	8.78	9.7	7.6	3.1	12.3	11.0	12.0	9.2	7.8	8.6	8.2	6.1
167	155	154	216	255	195	557	679	707	214	210	193	104	112	169	167	188	372
24.5	10.5	12.5	14.2	14.0	44.0	4.5	8.5	14.0	10.0	17.5	13.0	6.8	5.8	13.0	15.5	15.5	9.0
65.0	78.0	76.5	80.8	78.5	53.0	71.5	62.5	55.0	82.5	71.0	72.2	65.8	62.6	50.4	54.0	61.5	52.5
	2.0	1.0							0.5		1.2	2.2	2.4	0.8		0.5	
	4.5	2.5	1.8	2.5	1.0	4.0	5.0	1.0	4.5	5.5	5.0	6.2	8.8	7.0	6.0	2.5	8.0
						0.5						1.8	6.0	8.0	6.5	6.5	23.0
7.5	5.0	6.5	2.8	5.0	1.0	15.0	21.5	29.0	2.5	5.0	7.0	16.0	13.2	20.2	15.5	13.5	7.0
3.0		1.0	0.4		1.0	4.5	5.0	1.0		1.0	1.6	1.2	1.6	0.6	2.5		0.5
	1.0		0.4		1.0	1.0	0.5					0.2					
			101							24	23	12	55				
			33							None	None	None	33				
		2.2	3.4	0.4					0.5	0.2	1.0					0.2	1.2
1.2		6.2	3.0	2.6					10.0	1.2	1.6	1.0	0.6	1.8		0.6	6.4
		0.2								0.2				0.2			0.8
7.4		27.0	13.4	20.6					20.0	28.0	27.6	19.6	18.4	46.4		25.8	39.0
		0.4	0.2								2.0			2.0		0.4	0.6
0.8		24.4	66.2	21.6					5.25	45.8	40.4	53.6	15.8	13.0		11.6	8.6
		0.4	0.2	1.8						1.4	0.8	2.2	1.2	0.6		0.2	0.4
		2.2	0.6	0.6						0.2		0.2	0.6			0.2	0.2
		2.0	0.2	0.2						0.4			1.0	0.2		0.4	0.4
2.4		19.6	3.8	1.6					2.25	11.0	10.2	1.6	44.0	16.4		38.0	11.0
		0.8	0.8	0.2						2.0	5.0	3.8	3.0	2.4		1.2	0.8
53.0		13.0	7.2	47.8					60.0	8.8	8.4	14.6	10.2	10.6		14.2	18.6
											1.0	1.0	0.6	0.6		0.4	
35.0		0.2		1.2					1.75		0.8	2.2	2.6	2.0		4.2	10.6
0.2		0.4	0.6	0.8							0.8		0.2	0.6			0.8
		0.6	0.2								0.4	0.2	0.6	3.0		2.6	0.4
		0.4	0.2	0.6					0.25	0.8			1.2	0.2			0.2
3.91: 1.00		2.55: 1.00	18.78: 1.00	19.58: 1.00					15.88: 1.00	6.62: 1.00	7.19: 1.00	42.44: 1.00	0.78: 1.00	3.85: 1.00		1.00: 1.00	4.91: 1.00

skin, freely movable in the subcutaneous tissue, and were occasionally fixed to the underlying structures. The thoracic lymph nodes measured from 1.5 to 5.5 cm. and often encircled and compressed the trachea, bronchi, and esophagus. All the abdominal nodes were increased in dimensions, measuring from 1.5 to 9.0 cm. in diameter, with the greatest enlargements occurring in the sublumbar and mesenteric groups. The lymph nodes were usually discrete although adjacent ones sometimes appeared fused and matted together. They were a grayish tan and were moderately firm with a tense capsule. On sectioning a pale, watery fluid was often expressed. Some nodes cut with difficulty due to the softness of the nodal tissue. In several cases they were very soft and in 2 animals a few nodes were purulent.

Spleen. With the exception of those of 2 dogs, the spleens were greatly enlarged and measured from 23.5 to 41.0 cm. in length, 5.0 to 10.5 cm. in width, and 2.0 to 4.0 cm. in thickness. The edges were rounded and the capsules appeared tense and thinned. In 8 cases the spleens, on section, had the color, consistency, and appearance of raspberry jam, with innumerable spherical, pale gray nodules measuring from 0.2 to 0.3 cm. scattered throughout the pulp. In 3 spleens the pulp was a pale red, soft, and with similar nodules. In 2 animals (1 lymphocytic and 1 lymphoblastic) the spleens appeared normal. Several infarcts were present in 3 spleens. The enlarged spleens revealed an absence of the normal trabecular and pulp structure. There were several grayish tan nodules varying from 2.5 to 5.0 cm. in diameter in the subcapsular region in 2 spleens of the mixed cell type.

Liver. In 10 animals the liver was enlarged and in 3 instances it extended below the umbilicus. The edges were rounded and the surface was usually a light mottled red. The portal markings were rendered more prominent by innumerable minute, grayish nodules of pin-point to pin-head size, or by irregular, fine, grayish streakings.

Lungs. In 6 cases the lungs were congested and edematous. In 2 animals there were focal lesions of bronchopneumonia and in 5 they appeared normal.

Other Organs. In 1 case the posterior mammary glands showed areas of necrosis and purulent exudate. In 2 animals there were ascites, hydrothorax, and hydropericardium. In 1 case the small intestine just posterior to the duodenum was involved in a lymphomatous mass 7.6 cm. long and 5.1 cm. wide, with the intestinal lumen 0.5 cm. in diameter. The walls of the gallbladder were thickened in most instances. In all dogs the tonsils were enlarged, reddened, and protruded from their fossae. The membranae nictitantes were thickened and proptosed in 4 cases. Bone marrow examination was restricted to the ilium,

sternum, and ribs. Such marrow was a reddish color. The other tissues and organs showed no changes characteristic of the lymphomatous state.

Microscopic Observations

In general there were little, if any, differences among the various cellular types as far as the histologic involvement of the organs was concerned. All forms will therefore be considered together and any noteworthy variations will be mentioned.

Lymph Nodes. In most instances there was complete loss of architecture of the nodes so that the normal structure of follicles, trabeculae, and sinuses was over-run, obscured, and replaced by diffuse infiltrations of lymphoma cells. The capsule and perinodal fat were usually heavily infiltrated although in some nodes this involvement was light or moderate. These findings were also noted in nodes of normal size and in those slightly enlarged. The sinuses were sometimes persistent, particularly those of the medulla, and usually contained lymphoma cells. They were occasionally congested or edematous and fewer cellular elements were present. Infrequently there were focal areas of congestion and hemorrhage together with golden-yellow pigment (hemosiderin). Considerable hemorrhage involved several nodes of one animal. In most cases, one or more nodes showed focal areas of necrosis with polymorphonuclear leukocytic collections that were extensive in the definitely purulent nodes. Considerable variations were noted in the different cases and in the lymph nodes of the same animal in respect to the number of mitoses and of macrophages. Extramedullary hematopoiesis did not occur and an occasional megakaryocyte was seen among the infiltrations. Reticulum stains disclosed a relatively profuse, irregular argyrophilic network that encompassed groups of cells with no intercellular distribution (Figs. 11 to 13). In regions the reticulum was sparse, particularly in cases of the lymphoblastic and lymphosarcomatous cell types. The veins, lymph vessels, and rarely the arteries often showed mural infiltrations of lymphoma cells that were occasionally intravascular.

Dog 7760 deserves special comment as the lymph nodes regressed in size after the initial enlargement. The normal structure persisted despite the replacement of the lymphatic tissue with lymphoblasts. In two small nodes large numbers of lymphoblasts surrounded distinct follicles with secondary nodules.

The lymph nodes secured for biopsy were identical with those obtained at necropsy.

Spleen. The different spleens showed changes that could be classified into several categories irrespective of the lymphoma cell type. These

varied from no microscopic alterations (1 of lymphocytic type) to an organ that histologically resembled a lymph node (1 of mixed cell type). Between these extremes the spleens showed varying degrees of lymphomatous involvement. In practically all cases the capsule was markedly thinned and the trabeculae were reduced relatively in number. The lymphoma cells appeared as smaller and larger irregular nodules or as diffuse masses that often obscured and obliterated the usual architectural landmarks (Fig. 15). The malpighian corpuscles were generally absent although in some instances they were replaced by lymphoma cells. There were occasional small areas of necrosis in the central portions of the lymphoma cell groups. Between the nodular masses the pulp was often congested, hemorrhagic, and contained variable numbers of polymorphonuclear leukocytes and lymphoma cells, occasional plasma cells, histiocytes, monocytes, and frequently many megakaryocytes. The amount of hemosiderin, which is considerable in normal animals of this age group, was decreased in all cases except 2. In 5 spleens there were widespread areas of extramedullary myelopoiesis that consisted largely of myeloblasts, myelocytes, and band forms, a finding confirmed by the presence of these cells in imprints of the same spleens. In addition a few scattered areas of erythrocytogenesis occurred in 1 spleen of this group. The macroscopic presence of infarcts in 3 cases was confirmed by the histologic examination. The trabeculae and walls of the trabecular blood vessels commonly showed lymphomatous infiltrations with many cells assuming an intravascular position (Fig. 16). The splenic imprints contained, in addition to the cell types observed in the sections, relatively large numbers of pathologic lymphocytes that were identical with those noted in the peripheral blood.

Liver. In all cases there were portal lymphomatous infiltrations consisting of small and large irregular masses of perivascular distribution that involved from 10 to 70 per cent of the entire liver tissue (Fig. 14). The cells frequently invaded the walls of the veins and were occasionally intravascular although the arteries and bile ducts were normal. In addition, small lymphoma cell groups sometimes occurred in the sinusoids and around the central veins. In 4 cases there were moderately extensive areas of myeloid metaplasia. Megakaryocytes were commonly present in the capillaries. In the dog with jaundice, bile pigment occurred in the bile capillaries. The liver cells were well preserved although they occasionally showed mild fatty changes and were sometimes atrophic in the regions of heavy infiltrations. The sinusoids were congested and contained large amounts of hemosiderin

in several cases. In all animals there were heavy subcapsular infiltrations of lymphoma cells. An interesting observation previously described [49] is the reduced number of normally present intranuclear crystals in the liver while those of the kidney are in the normal range.

Gallbladder. The lamina propria, muscularis, and perimuscular connective tissue layer were heavily infiltrated with lymphoma cells while the mucosa and serosa were normal (Fig. 18).

Kidney. The recognition of lymphomatous infiltrations in the kidney is complicated by the fact that the dog frequently suffers a spontaneous interstitial nephritis [50] in which lymphocytic cells appear in the interstitial tissue. This disease is particularly common in animals of the age group in which malignant lymphomas occur. In interstitial nephritis, however, plasma cells occur, mitoses are rare, and there is often some fibrous connective tissue proliferation. In 5 cases (lymphoblastic and mixed cell types) there were several lymphomatous nodules of varying size in the corticomedullary junction and subepithelial pelvic connective tissue, and smaller perivascular collections in the cortex. The cellular collections were usually well localized and not extensive. The glomeruli, tubules, and blood vessels showed no alterations attributable to the infiltrations. Leukostasis was absent and in 3 kidneys there were several small areas of myeloid metaplasia.

Prostate. In older dogs the prostate often shows subacute and chronic inflammatory processes associated with lymphocytic infiltrations. As in the kidneys, recognition of lymphoma cells is difficult due to the pre-existent inflammatory cells. In the 6 male dogs on which autopsy was performed, lymphomatous infiltrations, usually as irregular nodules and less often as diffuse groups, occurred in the interstitial tissue in 2 cases (1 lymphoblastic and 1 mixed cell type).

Genital Organs. The testis, epididymis, and penis, and the vulva, vagina, uterus, and ovaries were free from lymphoma cells with the exception of the ovaries of 1 animal. In this dog there were diffuse nodular masses of lymphoma cells in the medullary portions of both ovaries.

Tonsils. In all cases the tonsils showed widespread infiltrations so that the normal structure was obscured (Fig. 19). The epithelial layer was flattened and lymph follicles were absent. Plasma cells, small lymphocytes, and neutrophils occurred in lesser numbers but macrophages were plentiful. The cellular infiltrations did not involve the mucous glands in the peritonsillar region.

Membrana Nictitans. The nictitating membrane of the dog normally contains distinct lymph follicles. In animals in which the third

eyelid was protruded, the follicular structure was replaced by diffuse infiltrations of lymphoma cells. Similar infiltrations were sometimes present in those eyelids that were not grossly enlarged.

Gastrointestinal Tract. Only 1 case (lymphocytic type) showed a gastrointestinal lymphomatous mass and that has already been described grossly. Microscopically, the intestinal coats were heavily invaded so that the normal structure was obliterated. The intestinal glands were fewer and the epithelium was ulcerated in areas. Despite the absence of macroscopic changes, Peyer's patches were usually replaced by lymphoma cells although there was no invasion of the neighboring structures. Changes characteristic of pseudoleukemia gastrointestinalis were not observed.

Pancreas. Lymphomatous infiltrations of the pancreas occurred in 5 cases of the lymphoblastic and mixed cell types. The cellular collections consisted of small, irregular nodules, principally confined to the interlobar connective tissue, that only occasionally infiltrated the intra-acinar connective tissue. The lymphoma cells were usually perivascular, often encircled ducts and ganglia, and sometimes occurred in the peripancreatic fat.

Lungs. In all cases the lungs showed more or less widespread, small and large nodular formations of lymphoma cells that were perivascular and peribronchial in distribution (Fig. 17). The vascular and bronchial walls were often invaded and lymphoma cells frequently occurred in their lumina. The interalveolar septa and alveolar lumina contained isolated lymphoma cells. Megakaryocytes were regularly present. The parenchyma was usually normal although congestion and edema and, in 2 cases, focal areas of bronchopneumonia occurred.

Adrenal. The adrenals showed lymphomatous infiltrations of various degrees in 9 cases. There were frequent perivascular nodules in the capsule and periadrenal fat that sometimes enveloped nerves and ganglion cells. Small cellular collections commonly occurred in the cortex, particularly in the zona reticularis and less often in the other zones. Widespread areas of myeloid metaplasia, limited to the fascicular and reticular zones, were present in 2 cases.

Eyes. In the dogs with exophthalmus there were large masses of lymphoma cells in the orbit that surrounded the optic nerve, muscles, and blood vessels but did not invade the periorbital tissues.

Bone Marrow. As previously stated, discrepancies were noted between bone marrow sections and the aspirated marrow concerning lymphomatous involvement. In the 2 animals in which lymphoma cells were absent, the marrow was hyperplastic in one and hypoplastic in the other. In the remaining 11 dogs the marrow showed scattered nodules

of lymphoma cells with a more diffuse infiltration in only 1 case. Surrounding the nodules were many megakaryocytes, few erythroid cells, and an extensive hyperplasia of myeloid cells (Fig. 10). The explanation for the discrepancies in the aspirated marrow probably lies in the fact that the biopsy needle may miss the nodules. Conversely, the needle may be inserted into a nodule so that numerous lymphoma cells will be aspirated and an erroneous high count obtained. The evidence indicates that the sectioned material gives a more accurate picture of the lymphomatous infiltrations in the bone marrow. Partial absorption of bone spicules, erosion of bone with extension to the periosteum, invasion of parosteal structures, and osteosclerosis were absent in our cases.

Other Organs. Microscopic examination of all other organs, including the brain and spinal cord of 3 animals, revealed no lymphomatous infiltrations.

Discussion

In considering a suitable designation for the animal disease, several possibilities were weighed. Lymphoblastoma was rejected because there is no substantial evidence that these tumors arise from embryonal cells.[51] Pseudoleukemia in the original sense of Cohnheim [52] would be suitable although this term is often used without any specific meaning and includes Hodgkin's disease, lymphosarcoma, and many others. Lymphadenosis signifies a glandular structure and the generalized lymphosarcomatosis of Kundrat [53] shows more regional involvement with less frequent spread to the spleen, liver, and bone marrow. Sternberg's leukosarcoma [54] is objectionable as the blood changes are absent. Gall and Mallory's term,[47] malignant lymphoma, appears the most suitable despite its relative noncommitment regarding the detailed histologic alterations. This criticism is tempered, however, by considering the cytologic structure of the predominant cellular types.

Although numerous studies have been made of the lymphomas in dogs, there have been few, if any, attempts to classify these diseases from a cytologic viewpoint. The impression received from a study of the literature is that some lymphocytic cell is involved, often without any qualifying statements concerning its detailed structure. In man, on the other hand, many cytologic classifications of the lymphomas have been proposed, largely from studies of paraffin-imbedded, sectioned material. When comparisons are made of the same material prepared as imprints stained with the May-Grünwald-Giemsa stain, certain discrepancies are observed attributable to the definite loss of cellular details that the sectioned tissue undergoes in the fixing, dehydrating, and embedding processes. The advantages of dry imprint

preparations have been summarized by Kirschbaum and Strong [55] as follows: (1) All types of leukemic cells are present, rather than only those which gain entrance into the blood. (2) The cells are more easily classified than in sections. (3) The same criteria for morphologic identification of cells can be used here as in blood cells. The dry imprint method has been largely used by workers with experimental leukemia and only occasionally in investigations of human leukemia. Certain cells, such as the mature small lymphocytes, are equally recognizable in imprints and sections. When immature lymphocytic cell types are considered, the section method appears to be inaccurate unless imprints are studied simultaneously and used as a basis for comparison. For example, in our Figures 5 and 6 of sectioned lymph nodes of the lymphoblastic type, the cells resemble those which Gall and Mallory [47] term the stem cell type, and which Ehrlich and Gerber [48] denote as the reticular type. The imprint material of the same nodes indicates that these cells have the accepted morphologic characteristics of lymphoblasts and are probably not stem cells or reticular cells in the usual interpretation commonly applied to these terms. The lymphoblastic type of Gall and Mallory [47] does not resemble our lymphoblast and is probably a form intermediate between the lymphoblast and the mature lymphocyte. In our own material likewise discrepancies occur unless imprints are compared with the sections. The imprints of the lymphoblastic type shows cells (Fig. 1) which are entirely different from those of the lymphosarcoma cell type (Fig. 2). Sections, however, as depicted by Figure 5 of the former and Figure 7 of the latter type, are practically identical.

While conclusions derived from a study of animal material may have limited application to the human disease, the evidence strongly suggests that in the lymphomas, at least, there is definite loss of cytologic details in sectioned tissue. Such material should therefore be used as a means of cellular classification provided its limitations are known and preferably if lymph node imprints are studied simultaneously. It is essential to use absolutely fresh tissue, preferably surgical specimens, in preparing imprints, as autopsy material is unsuitable due to the associated autolysis.

In view of the hematologic findings, it is difficult to justify the use of the term "leukemia" in the canine disease. In our series and in the great majority of cases reported in the literature, the blood changes were not comparable to those occurring in human leukemia. The most characteristic blood alteration in addition to the anemia consisted of a nonspecific leukocytosis resulting from increased numbers of nonsegmented and segmented neutrophils. This blood picture frequently

has been considered as an expression of myeloid leukemia (Weil and Clerc,[9] Wirth,[18, 25] and others) and these authors have been extensively quoted to support the opinion that the dog develops this disease. Our blood studies, together with those reviewed in the literature, suggest that no bona fide instances of myelogenous leukemia have been described in this species. The mechanism for the leukocytosis is a speculative question and several possibilities can be considered. The presence of leukocytosis with malignant tumors in animals and man is well known and is usually attributed to the associated ulcerative and inflammatory processes.[51] Lewis [56] and others have demonstrated that the growing malignant tissue of dibenzanthracene tumors in mice produced an increased number of polymorphonuclear cells in the peripheral blood. Leukocytosis is often found in invasion of the bone marrow by malignant tumors. In the canine malignant lymphomas the leukocytosis probably results from a combination of these factors; namely, the marrow involvement, the necrosis in the lymphomatous tissue, and the relatively rapid growth of lymphoma cells. The hyperplasia of myeloid cells in the bone marrow adequately explains the neutrophilic leukocytosis encountered in the peripheral blood. Although the replacement of normal marrow by lymphoid foci is considered an important factor in the production of the accompanying anemia in man,[57] this explanation cannot be unreservedly applied to the canine disease. In the dog, the suppression of erythrogenesis results from the myeloid hyperplasia and only partially from the lymphomatous infiltrations which are both moderate and focal in extent. The anemia is probably similar to that occurring in many malignant tumors.

A consideration of the histologic alterations in the malignant lymphomas of dogs presents features that are of interest to the comparative pathologist. The aggressive tendencies, such as capsular and perinodal infiltrations and the nodular cellular masses in the different organs, point toward lymphosarcoma. On the other hand, the almost universal involvement of the spleen, liver, and bone marrow, in addition to diffuse cellular infiltrations, are not the typical findings that one usually associates with lymphosarcoma. While it is well known that the lymphomas are often difficult to separate microscopically and that numerous transitional forms occur, the malignant lymphomas in dogs, as here described, appear to be intermediate forms, irrespective of the cell morphology, between the pseudoleukemia of Cohnheim [52] and the lymphosarcomatosis of Kundrat.[53] The disease seems to partake of the histologic characteristics of both affections and in this respect resembles similar transitional types in man.

It might be of interest to consider the relative frequency of the

lymphomas in general as compared with other neoplasms occurring in dogs. Specifically, solitary lymphomas are relatively uncommon; in fact, in the experience of one of us (F. B.), only 1 such tumor was observed in a series of 196 neoplasms of all types. This growth was a primary reticulum cell lymphosarcoma localized in the right prescapular lymph node. The so-called lymphomata so frequently present in the spleens of older dogs cannot be placed in this category as they undoubtedly represent nodular hyperplasia and are not true neoplasms. The splenic "lymphomata" are analogous to the nodular hyperplasia occurring in the adrenals, liver, and pancreas of aged animals. The malignant lymphomas can thus be considered the most common tumor-like enlargements of the lymph nodes in dogs.

The fairly extensive areas of extramedullary myelopoiesis in 5 animals that involved the spleen, liver, kidneys, and adrenals are of interest. Such findings appear to be common in the canine malignant lymphomas as judged by the many reported cases of so-called myelosis with which the myeloid metaplasia has been confused. In man, on the other hand, extensive myeloid metaplasia is exceptional and has led to the question of a mixed or combined (myeloid and lymphatic) leukemia.[57] In dogs, however, the concept of a myeloid or mixed leukemia cannot be seriously entertained on the basis of the ectopic myelopoiesis in view of the universal presence of cells of lymphocytic type in the different organs. The heterotopic collections of myeloid cells probably arise from a combination of factors; namely, compensation for the partial replacement of myeloid tissue in the bone marrow by lymphoma cells and, as Lewis [56] has demonstrated in experimental tumors, the specific biologic features of some neoplasms that stimulate the formation of myeloid metaplasia. The extramedullary myelopoiesis occurred in the lymphocytic and mixed cell types only.

From the clinical point of view, the duration and course of the disease was subacute with little variation in the different cellular types. The usual subdivision into acute and chronic cannot be applied to our cases on the basis of cellular morphology or of duration of the disease.

Summary

1. The designation, malignant lymphoma, has been applied to a systemic disease of dogs in which the lymph nodes, spleen, and liver were usually enlarged. Microscopically, diffuse and nodular cellular infiltrations involved these organs as well as the bone marrow, adrenals, lungs, kidneys, prostate, tonsils, third eyelids, gallbladder, pancreas, and Peyer's patches. On a cytologic basis, the predominant cellular

types were classified into four distinct groups: lymphoblastic, lymphosarcoma cell, mixed cell, and lymphocytic.

2. Cellular identification as judged by dry imprints and sections of lymph nodes indicated greater accuracy for the former method. Therefore, the dry imprint method should be employed in classifying the lymphomas cytologically.

3. The peripheral blood showed anemia and usually a polymorphonuclear leukocytosis and is therefore not comparable to the true leukocythemia in human malignant lymphomas. Thus the term, leukemia, is inadmissible for the canine disease.

4. The widespread extramedullary myelopoiesis that is not uncommon has been frequently erroneously reported as myelogenous leukemia in the literature. Actually, no unquestionable cases of this disease have been described in dogs.

5. Clinically, the disease was subacute, irrespective of the predominant cellular type, in contradistinction to the usual findings in man.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

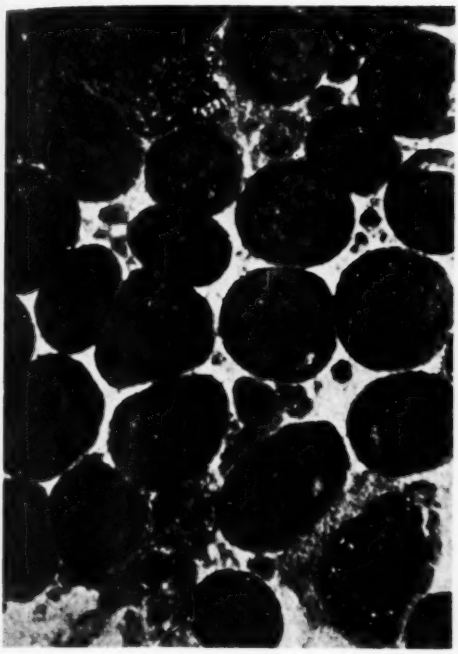
PLATE 117

FIG. 1. Lymph node imprint (case 8847, lymphoblastic type). The lymphoblasts have leptochromatic nuclei with large, prominent nucleoli. May-Grünwald-Giemsa stain. × 1320.

FIG. 2. Lymph node imprint (case 8313, lymphosarcoma cell type). The cells in the photomicrograph have a relatively mature nuclear structure and the prominent nucleoli are surrounded by a rim of deeply staining perinucleolar chromatin material. In other cells of this imprint the nuclei were more immature but the nucleoli were similar. Brilliant cresyl blue and Wright's stain. × 1320.

FIG. 3. Smear preparation of lymph node macerated in the serum of the same animal (case 1474, mixed cell type). The photomicrograph depicts four mature small lymphocytes, one lymphoblast (arrow), and four younger lymphocytes without nucleoli. Brilliant cresyl blue and Wright's stain. × 1320.

FIG. 4. Lymph node imprint (case 7838, lymphocytic type). The cells resemble the mature lymphocytes of the peripheral blood and contain pachychromatic nuclei without nucleoli. May-Grünwald-Giemsa stain. × 1320.



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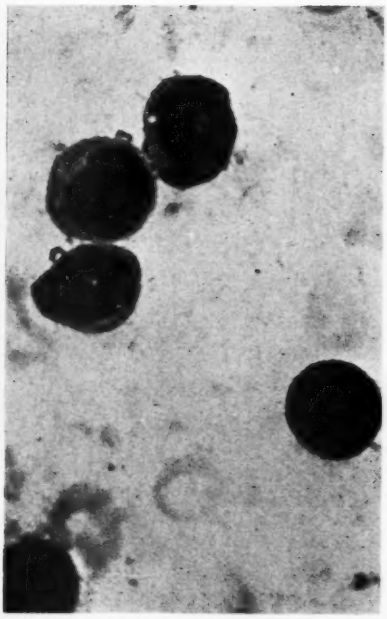

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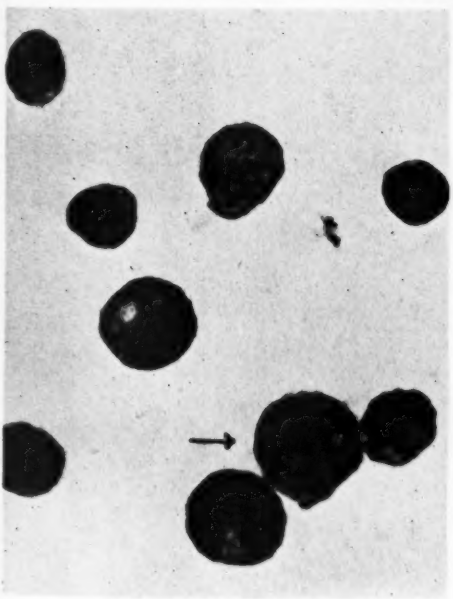

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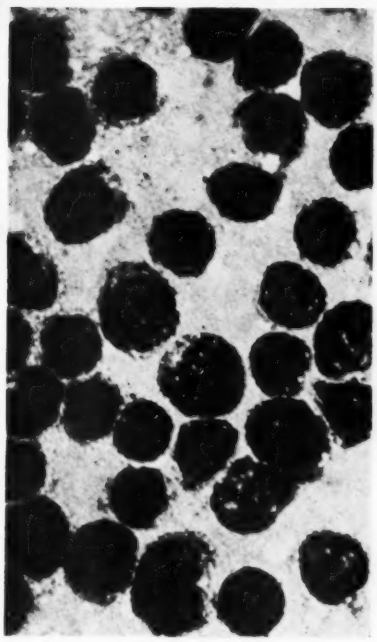

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PLATE 118

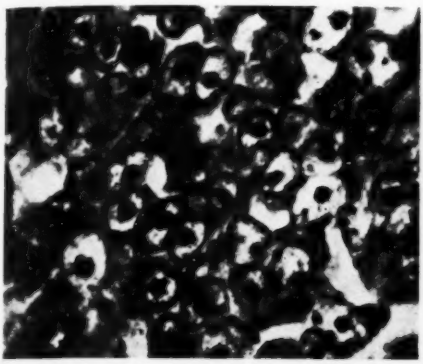
FIG. 5. Paraffin section of lymph node of the same case as shown in Figure 1. The lymphoblasts have vesicular nuclei and prominent single nucleoli. Dominici's stain. × 1320.

FIG. 6. Section of lymph node (case 6088, lymphoblastic type) in which there are multiple nucleoli and the same vesicular nuclear structure. The cytoplasmic boundaries are readily discernible as the cells are less densely packed in this field. Dominici's stain. × 1320.

FIG. 7. Section of lymph node of the same case as shown in Figure 2. From sections the lymphosarcoma cells in this figure and lymphoblasts of Figure 5 appear alike but differ in the imprints of the same cases as illustrated in Figures 1 and 2. Dominici's stain. × 1320.

FIG. 8. Lymph node section (case 7067, mixed cell type) showing lymphoblasts, mature small lymphocytes, and cells intermediate between these. Dominici's stain. × 1320.

FIG. 9. Lymph node section of the same case as shown in Figure 4. The cells are morphologically similar to mature lymphocytes as seen in sectioned material. Dominici's stain. ×1320.



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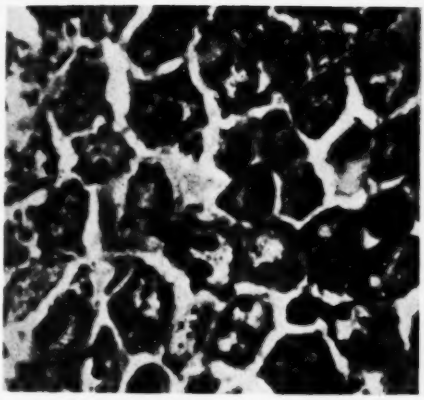

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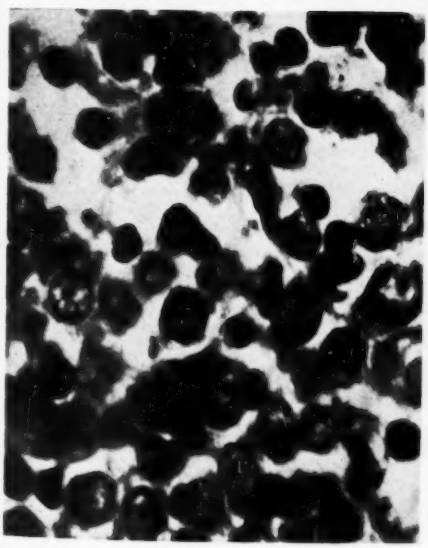

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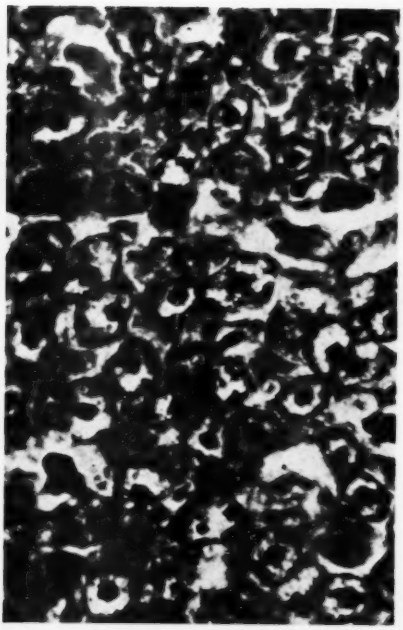

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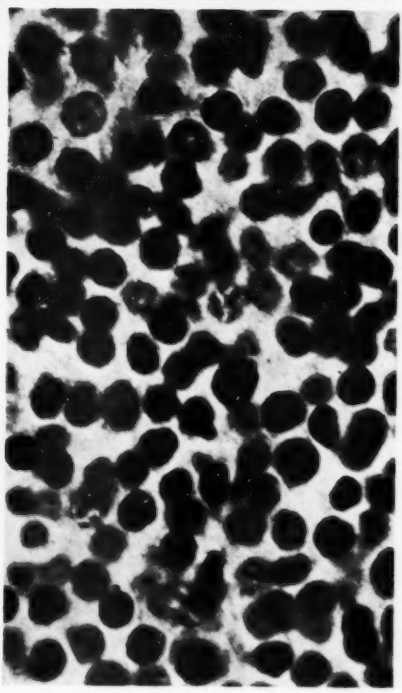

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FIG. 10. Bone marrow section of case 6088 (lymphoblastic type). The right half of the photomicrograph is occupied by a lymphomatous nodule consisting of lymphoblasts. To the left, myeloid cells predominate and part of a megakaryocyte is present in the extreme upper left corner. Hematoxylin and eosin stain. × 880.

FIGS. 11, 12, and 13. Reticulum stains of lymph nodes of cases 6088 (lymphoblastic type), 7067 (mixed cell type), and 7838 (lymphocytic type) respectively. The argyrophilic fibers are similarly distributed in all 3 cases. Wilder's reticulum stain. × 293.

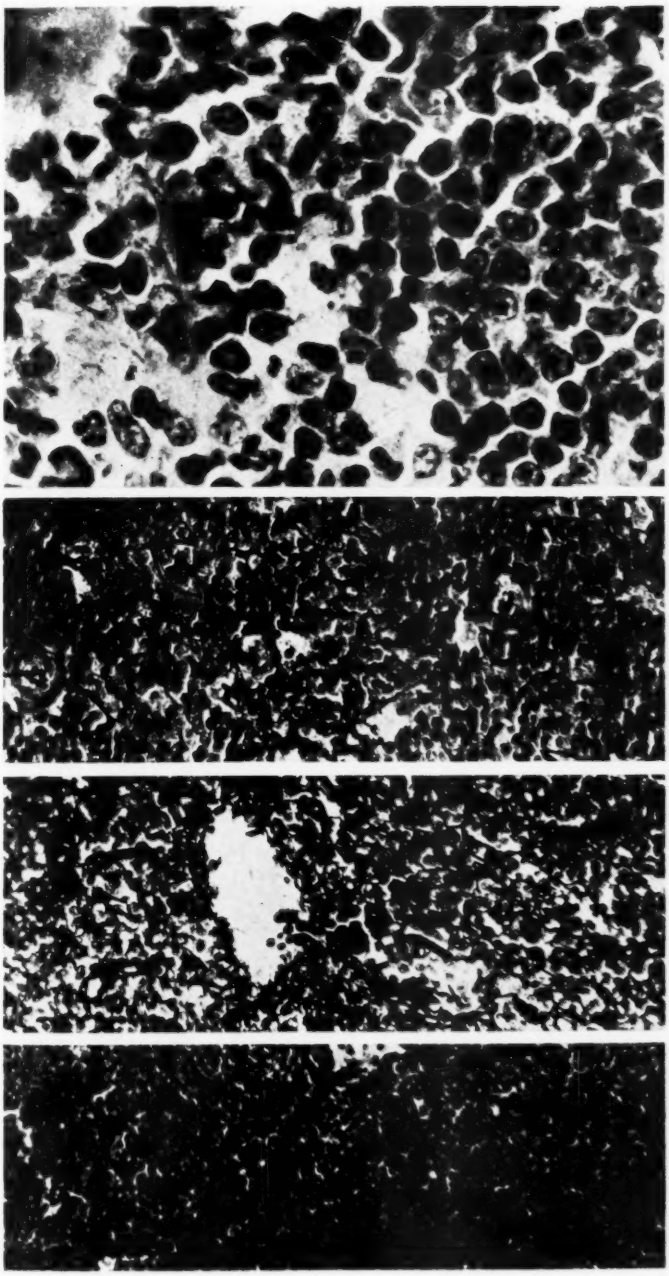

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PLATE 120

FIG. 14. Liver of case 7838 (lymphocytic type) depicting widespread portal and capillary lymphomatous infiltrations. In other cases the sinusoids were less extensively involved and the infiltrations were principally confined to the portal areas. Hematoxylin and eosin stain. × 88.

FIG. 15. Spleen of case 7760 (lymphoblastic type) illustrating a relatively diffuse infiltration of the pulp with lymphoma cells. Hematoxylin and eosin stain. × 88.

FIG. 16. Spleen of case 8847 (lymphoblastic type) with invasion of the trabeculae by cellular infiltrations. The lumen of the trabecular vessel contains a large lymphomatous nodule in addition to less compactly arranged cells. The dark staining granular material is hemosiderin. Dominici's stain. × 88.

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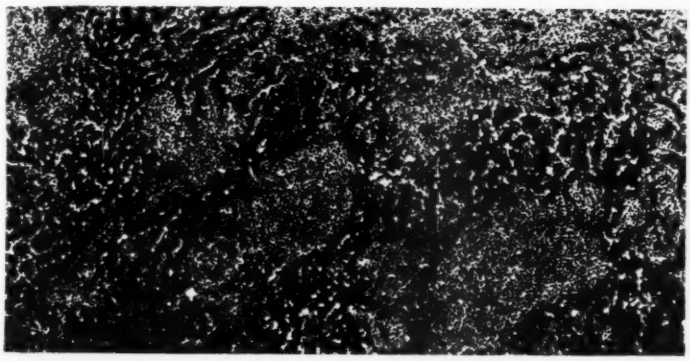

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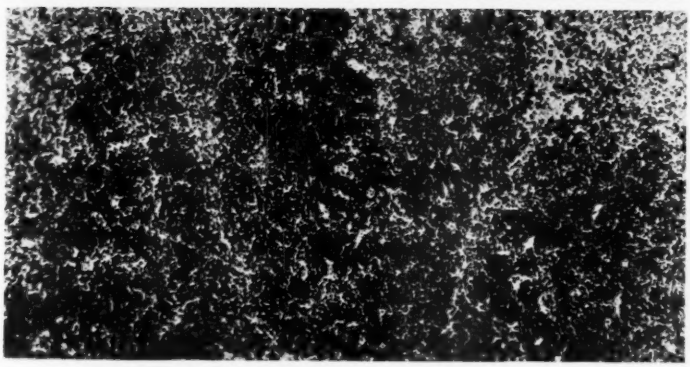

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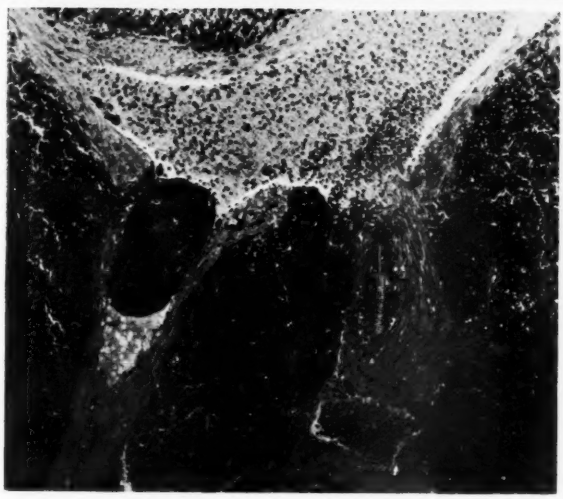

Bloom and Meyer Malignant Lymphoma in Dogs

PLATE 121

FIG. 17. Lung of case 8847 (lymphoblastic type) showing lymphomatous nodules and invasion of the bronchial walls in the right half of the photomicrograph. Hematoxylin and eosin stain. ×88.

FIG. 18. Gallbladder of case 1474 (mixed cell type) depicting diffuse lymphomatous infiltrations. The epithelium is normal. Hematoxylin and eosin stain. × 88.

FIG. 19. Tonsil of case 6088 (lymphoblastic type) with diffuse lymphoma cell infiltrations, among which there are numerous macrophages. Hematoxylin and eosin stain. × 88.

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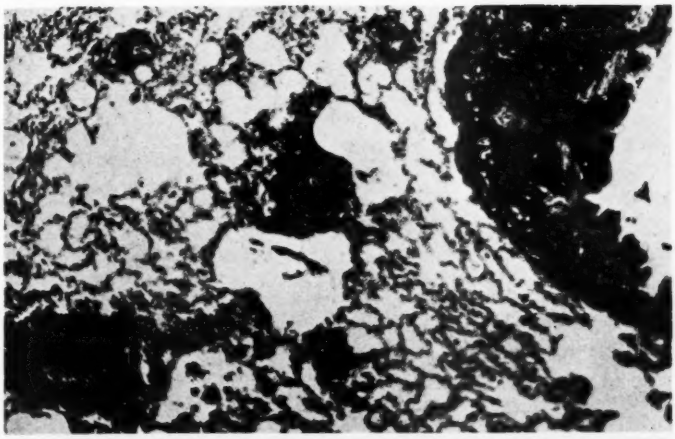

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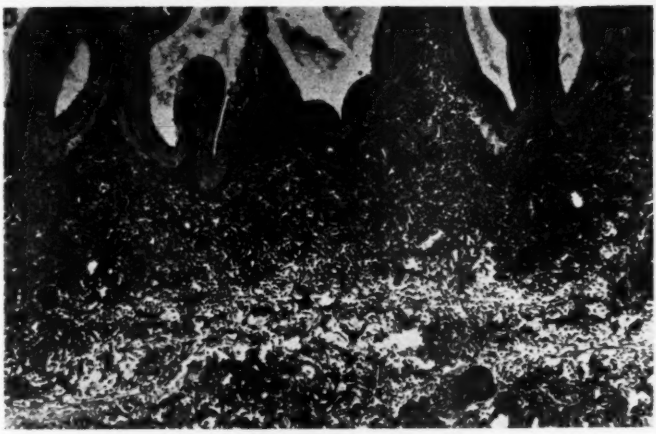

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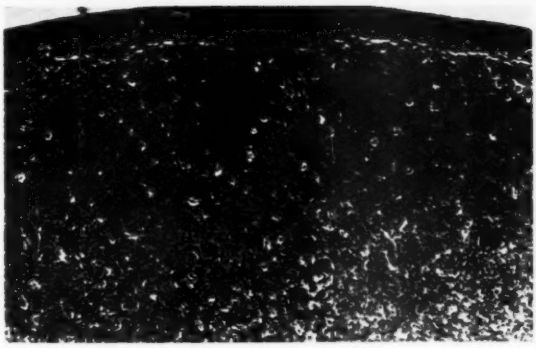

THE INTERNAL LESIONS IN BURNS WITH SPECIAL REFERENCE TO THE LIVER AND TO SPLENIC NODULES

An Analysis of 96 Autopsies *

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The assertion by some [1, 2] that hepatic necrosis is usual in fatal burns has been countered by others [3] with the suggestion that the hepatic necrosis is due to tannic acid which had been applied in treatment. A desire to settle this issue caused me to examine a series of autopsies which had been performed on patients who had died following cutaneous burns. This led to observations on the state of the splenic nodules in burns and to an examination of the adrenals and some of the other viscera of special interest. The splenic nodules have attracted attention ever since Bardeen [4, 5] described well marked focal areas of degeneration in them which he interpreted as being indicative of a toxemia of burns. Weiskotten [6] described changes in the adrenal glands.

The problem of the internal lesions of fatal burns has been complicated in recent years by the use of local applications of tannic acid and other escharotics, by the giving of sulfonamides, and by the use of plasma and other intravenous fluids. Autopsies and reports made several decades ago should be examined with special care as they may actually be more reliable than autopsies performed recently. Fortunately, a portion of the autopsies which I examined came from this earlier period.

Material

Analysis was made of the cases of burns which had been autopsied at Duke Hospital and at Johns Hopkins Hospital.†

Only those cases were selected in which burns or the complications of burns appeared to be the principal factor leading to death. In reviewing the cases at Duke Hospital, complete protocols were prepared and all microscopic sections and the clinical histories were thoroughly studied. In reviewing the cases at Johns Hopkins Hospital I examined slides of liver, spleen, adrenal, and kidney in all cases in which these sections were available, and made notes of each case, as indicated in the sample protocol which follows:

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Duke University.

Received for publication, July 25, 1944.

† Permission to examine the autopsy protocols and slides at the Department of Pathology at Johns Hopkins Hospital was secured from Dr. A. R. Rich, and to examine the clinical records at Johns Hopkins Hospital from Dr. Winford H. Smith.

Colored male, 3 years of age (autopsy no. 15592). Death 2 days after burn. Two-thirds to three-fourths of body surface involved. Gentian violet spray. Temperature and pulse rose very high. No infection demonstrated in sections of skin. Spleen showed early acute splenic tumor. Liver showed slight diffuse fatty change.

In interpreting the histologic changes, the duration of survival is of great importance (Table I), for when death occurs after only a few hours it is conceivable that cells have already been damaged physiologically without cytologically demonstrable evidence of such damage. On the other hand, when death occurs several weeks after burning, cells which were necrotic at first may have been replaced by regeneration. Also, burns may become infected and bacteremia may develop in patients who survive longer than several days, and the changes observed in the internal organs may be the result of the bacteremia and not of the

TABLE I

Duration of Survival in Fatal Burns

Death during:	No. of cases
1st day	37
2nd day	9
3rd day	9
4th day	4
5th day	0
6th day	4
7th day	0
2nd week	7
3rd and 4th weeks	12
Period beyond 4 weeks	14
Total	96

burn. Necroses in these older cases may be due to other complicating factors such as anemia and congestive heart failure.

Analysis of Table I showing duration of survival indicates that slightly more than one-third of the patients died during the first day, slightly less than one-third died during the remainder of the first week, and almost exactly one-third died after the first week. There were 26 cases in the most interesting period of all, that beginning with the second day and extending through the first week. It is during this period that deaths from burns occur with the least satisfactory explanations. Deaths during the first day are explainable on the basis of shock, especially when the burns are very severe, and deaths after the first week are usually explainable on the basis of complicating factors, but during the period from the second day through the first week primary shock has largely disappeared and infection usually has not developed.

Liver

Hepatic necrosis was not mentioned prominently in reports of fatal burns published before 1925, *i.e.*, before tannic acid was used on burns. In Schjerning's [7] table published in 1884 showing the occurrence of pathologic lesions following burns, the liver is not even listed. Dohrn [8] found no changes in the hepatic cells. McCrae [9] saw no necrosis in 12 cases. Marchand [10] stated: "The liver shows no essential finding. In high grades of blood destruction increased secretion of bile can occur, as in other types of hemoglobinemia." Weiskotten,[6] reporting 10 cases, found foci of necrosis of liver cells in 2.

Since 1925, attention has been called to severe hepatic necrosis in fatal burns, but apparently the severe grades of necrosis have been due to the tannic acid which was applied to burned areas. Vogt,[11] in 1929, reported 8 cases of fatal burns and found no necrosis of hepatic cells. He mentioned fatty change in these cells, and an increase and degeneration of leukocytes in the hepatic capillaries. In 3 of his cases death had occurred before 1925. There is no information as to whether tannic acid had been used in the treatment of the cases in which death had occurred after 1925.

In 1938 Wilson, Macgregor, and Stewart [1] described hepatic necrosis of severe grade in fatal burns following survival periods of from 2 to 12 days. (A photomicrograph of liver from one of their patients who died 71 hours after a burn shows extreme necrosis with only a rim of viable cells about the periportal areas.) Tannic acid had been applied to the burns in many of their cases. In 1939 Belt [2] described the hepatic necrosis following burns as similar to the hepatic lesions of yellow fever. In his 4 cases in which tanning with tannic acid had been employed, death occurred between the second and fourth days of survival. The necrosis is described as mid-zonal, but the illustration indicates that exceedingly few viable hepatic cells remain either about the central vein or about the periportal areas. Further evidence of the relationship between hepatic necrosis and the tannic acid therapy of burns [3, 12, 13] is summarized in an editorial in *The Journal of the American Medical Association*.[14] Wells, Humphrey, and Coll [3] were apparently the first to realize that the tannic acid was responsible for the hepatic necrosis rather than the burns, and they supported their view by producing hepatic necrosis in rats by subcutaneous injections of tannic acid.

Erb, Morgan, and Farmer,[15] in 1943, reported that of 27 patients tanned with tannic acid and dying in the period between the 3rd and 19th day, only 3 failed to show hepatic necrosis. The hepatic necrosis

illustrated by them is of extremely high grade, involving the hepatic lobule for nine-tenths of the distance from the central vein to the periphery. In strong contrast, no case of hepatic necrosis occurred in their 20 patients in the untanned group, 7 of whom died between the 3rd and the 14th days, a period in which necrosis was an outstanding feature in the tanned group; and no hepatic necrosis was seen by them in patients who had died before the introduction of tannic acid therapy for burns.

Observations

Necrosis was noted in the cases which I examined, but with relative infrequency, as indicated in Table II. Moreover, the necrosis was usually not at all severe. In only 3 instances was it as much as 2 plus,

TABLE II

Liver in Fatal Burns

Time of death in relation to injury	No. of cases with sections of liver	No. with necrosis	No. with fatty change
1st day	32	1 (±)	6 (+++, +, +, +, ++, +)
2nd day	9	1 (±)	2 (+, +)
3rd day	8	3 (±, +, +)	1 (+)
4th day	3	0	0
6th day	4	1 (±)	0
2nd week	6	1 (+)	0
3rd and 4th weeks	11	2 (+, +)	2 (++, +)
More than 4 weeks	14	4 (++, ++, ±, ++)	9 (+, ++, ++++, +++, +, +, ++, ±, +++)
	87	13	20

which indicated necrosis extending nearly one-half of the distance from the central vein to the periphery of the hepatic lobule. But all 3 patients had survived for more than 4 weeks, and necrosis could scarcely be thought of as the effect of the original burn, since cells originally rendered necrotic would have been removed unless, of course, a necrotizing influence from the burn continued to be present. Tannic acid had not been used in these cases.

In all 3 cases complications of the burns or other accessory factors explained the hepatic necrosis more reasonably than the direct effect of the burns. In the first case, a child of 1 year, there was purulent otitis media and thrombosis of cerebral veins. In the second case, a male, 66 years of age, there was thrombosis of periportal veins, pulmonary emboli, dilatation of the right side of heart, syphilis with gummata of lungs and testes and perforation of the palate, and bilateral lobular pneumonia. In the third case, a man, 52 years of age, a burn of the right arm extended to the bone and was treated at home for 11 days. Twenty-one days before death the patient was brought to the hospital

with thick exudate covering the arm. Phlebitis of the popliteal vein developed.

Five of the cases showed hepatic necrosis of a grade of 1 plus, indicating that necrosis did not extend more than a quarter of the distance from the central vein to the periphery of the liver lobule. Usually the extent was less. It occurred in 2 cases with death on the third day, in 1 case with death in the second week, and in 2 cases with death in the third and fourth weeks.

Analysis of necropsy and clinical data in these 5 cases indicated that in 1 case there was nothing but the burn to explain hepatic necrosis; in 1 case there were possible accessory factors, but the burn may have been the factor; and in 3 cases hepatic necrosis was explained best on complications such as infection or cardiac failure.

The following notes were made concerning the case in which there was nothing but the burn to explain hepatic necrosis:

> Colored male, 41 years of age. Death on third day following burn in December, 1925.
>
> Section showed central necrosis of the liver with polymorphonuclear neutrophils about the borders of necrotic cells. The central necrosis occurred in some areas of the section and not in others.
>
> The anatomic diagnosis was: Burns. Central necrosis of the liver.
>
> The clinical record indicated that the patient was burned over one-half of the body surface 3 days before death. There was no note indicating the presence of jaundice, and no evidence that tannic acid was ever applied, and there was a note that vaseline gauze had been used. The pulse rate was 100 to 130 per minute. Death occurred following urinary suppression.

Five cases showed probable hepatic necrosis, as indicated by a plus-minus sign. In the sections of liver from these cases there was mild condensation of the nuclear material in the cells closely surrounding the central veins, such as to suggest pyknosis, but no corresponding cytoplasmic change; or there was some other slight change of questionable significance.

In 2 of these 5 cases with equivocal necrosis there was no factor but the burn to account for the change noted microscopically.

When Table II is reconstructed with omission of the cases in which hepatic necrosis is better explained by complicating factors, it appears as in Table III.

Thus, hepatic necrosis of high grade did not occur as a direct result of burning. Necrosis of minimal grade was present in four instances in which the burn may have been the cause. It was noted with death on the first day in one instance (of plus-minus grade), and with death on the third day in three instances. Hence, minimal degrees of hepatic necrosis may apparently be caused by burns; and it seems probable that the changes observed were not present in the liver before the burns

occurred, as the nearly complete absence of necrosis in the large group of patients dying during the first day suggests.

How was the minimal necrosis produced? The untoward effects of therapeutic agents may have been responsible. Tannic acid was not applied in any of these 4 cases. (In another case, not included in the revised table, necrosis may have been due to tannic acid. This is discussed later.) Sulfadiazine spray was used in one. Plasma and other intravenous fluids might be responsible, since the older reports mention necrosis so infrequently. Other theories include the acute congestion of shock, absorption of toxic substances from the burned area, and throm-

TABLE III

Liver Necrosis in Fatal Burns
(Necrosis Due to Complicating Factors Excluded)

Time of death in relation to injury	No. of cases with sections of liver	No. with necrosis "due" to burn
1st day	32	1 (±)
2nd day	9	
3rd day	8	3 (+, +, ±)
4th day	3	
6th day	4	
2nd week	6	
3rd and 4th weeks	11	
More than 4 weeks	14	

bosis. No support for the last theory was obtained from microscopic study.

Hepatic Fat. Histologically demonstrable fat was noted most frequently in those who died on the first day and in those who died in the period beyond 4 weeks. When it was noted in those who died on the first day, the question arises as to whether the fat was present before the burn as a storage phenomenon or whether it indicates hepatic injury. This question cannot be answered without control studies. When the fat was noted in those who died in the period beyond 4 weeks, the question arises again as to whether the state of nutrition of the patient accounted for the fat or whether the fat indicated hepatic damage. In some instances in which the fat bordered the necrotic areas it seemed clear that it indicated hepatic damage, but in the numerous instances in which fat was present without necrosis the fat was not interpreted as an indication of hepatic damage.

Tannic Acid

Apparently the tannic acid therapy of burns never had any great vogue at Johns Hopkins Hospital or at Duke Hospital. Some of the Hopkins cases were autopsied before the advent of the tannic acid treatment, and it was possible to study these early cases with certain

knowledge that tannic acid was not utilized. Twenty-eight of the Hopkins cases occurred before 1924. Davidson's [16] paper, "Tannic Acid in the Treatment of Burns," appeared in August, 1925; and the cases which he studied occurred in and after May, 1924. Of the 28 cases, sections of liver were present in 21, all of which were normal except 2. The protocols of the 7 cases without sections indicated the probable absence of hepatic necrosis in these cases also. Of the 2 cases, 1 case, previously mentioned, showed 2 plus hepatic necrosis probably associated with secondary infection of the burn, death occurring 32 days after a small burn. In the other case there was questionable necrosis; and confluent lobular pneumonia with toxemia, or with cardiac failure, offered as satisfactory an explanation as did the burns for the slight changes noted in the liver. Hence, in a series of 28 cases from the period when tannic acid was not used there was no case of hepatic damage due to the direct effect of the burns.

In the cases at the Johns Hopkins Hospital after 1924 it was not determined in each instance whether tannic acid had been used, since analysis of the clinical records was impracticable. But in all those cases in which hepatic necrosis was observed microscopically the clinical record was thoroughly reviewed in attempting to make certain whether tannic acid had been used. In only 2 of the 13 cases of Table II which showed necrosis microscopically had tannic acid been used. In 1 of these, the child was treated with tannic acid compresses. He died on the second day and no condition other than the burns was discovered. The minimal necroses in this case may have been due to the absorption of tannic acid. In the other case a girl of 9 years was treated with tannic acid over a burn of more than half of the body surface sustained 44 days before death. The minimal necrosis of the liver could not have been due to the tannic acid, because of the long interval of time; and there were other factors, such as profound anemia, which could have produced the hepatic change. Hence there is one case in this series in which hepatic necrosis may have been produced by tannic acid.

The problem may be approached, also, by determining what the liver showed when it was known that tannic acid had been used. This was true in at least 2 of the cases at the Johns Hopkins Hospital. In 1, previously mentioned, death occurred on the second day. The equivocal necrosis may have been due to tannic acid. In the other, tannic acid had been applied to second degree burns of the back and buttocks of a colored boy of 18 months. Death occurred 12 hours or so after the burn. The liver appeared normal, but with such early death the general effects of tannic acid would probably not be histologically apparent.

Tannic acid had been applied to two of the burned patients autopsied at Duke Hospital. Death occurred 30 days and 44 days after burning and no necrosis or scarring was noted in either case. At such long intervals after the burn any original necrosis might have been replaced by regenerated liver cells.

Concerning tannic acid, then, the present series gives little information except in comparison with the series of others, such as those of Wilson, Macgregor, and Stewart,[1] Belt,[2] or Wells, Humphrey, and Coll,[3] in which tannic acid was used extensively and in which necrosis of high degree was noted when death occurred during the first few days after the burns were received. The comparison indicates that hepatic necrosis in the cases of these authors was the result of the tannic acid treatment rather than of the burns. Experimental studies of Wells, Humphrey, and Coll, of Baker and Handler,[17] and of others, show clearly that hepatic necrosis can be produced regularly in experimental animals by the application of tannic acid to a wound or by subcutaneous injection of tannic acid.

Splenic Nodules

Bardeen [5] described swelling and necrosis in the lymphoid nodules of the lymph nodes, peripheral lymphoid tissue, and spleen which he considered characteristic of burns and indicative of toxemia, just as similar changes in diphtheria were indicative of toxemia. Regarding the spleen he stated:

> "The Malpighian bodies become enlarged from the swelling of their cells and from the subsequent degenerative and necrotic process which begins at the centre. Single cells and small groups of cells may be seen degenerating in all parts of the pulp, but well-marked focal areas of degeneration are confined to the centres of the Malpighian bodies."

These observations were made on the organs of five children who had been fatally burned. The children varied in age from 16 months to 8 years. The time elapsing between burning and death varied from 4 to 9½ hours. He commented on the fact that in the child who died soonest, and only 4 hours after the burn, the lymphatic lesions were well marked, but he did not suggest that the changes might have been present before the burn was sustained. In a footnote Bardeen [4] stated:

> "Since working up the cases described above I have had the opportunity of studying the tissues from the bodies of two adults whose death was due to superficial burns. In each case lesions similar to those described above were found, but the lesions seemed less marked in the lymphatic tissue of the adults than they were in those of the children."

McCrae [9] did not find the constant presence of focal necrosis in the lymphatic glands, upon which Bardeen laid considerable stress.

Dohrn [8] did not note in his cases the full development of the changes described by Bardeen. He recognized that Bardeen described the changes in children and he questioned whether the changes were specific for burns.

Weiskotten [6] reported on 10 cases of burns, 5 in children and 5 in adults. Regarding the spleen he stated:

"Microscopically, in all of the cases of less than 3 days' duration there were found rather characteristic lesions in the lymph nodules. . . . There was apparent necrosis of the cells of the germinal centers evidenced by karyorrhexis. Endothelial leukocytes . . . were phagocytic for the necrotic cells and for the lymphoid cells of the nodules. . . . In many instances this process continued until the lymph nodule was represented by a large central area filled with phagocytic endothelial leukocytes surrounded by a narrow rim of lymphoid cells. These lesions apparently developed very soon after the burns were received, and were evident in all of the cases of less than three days' duration. In the cases of more than three days' duration, there were areas corresponding in distribution to the lesions described in the earlier cases. These areas were relatively homogeneous and eosin staining with occasional vesicular nuclei. At the periphery of some of these were seen occasional cells resembling the endothelial leukocytes seen in the earlier lesions. These appearances suggest that the areas represent the earlier lesions in process of hyaline degeneration and resolution or repair."

Lubarsch [18] considered the changes in the lymph nodules to be the most constant finding in uncomplicated fatal burns. He noted that the change occurred in the great majority of instances in children, and pointed out that in children the lymphatic tissue reacts especially vigorously in the most varied diseases. Vogt [11] stated that in children the foci were regularly present. Fender [19] was interested in the changes in the lymphoid nodules as indicating toxemia. He mentioned the similarity of the changes in infections and intoxications such as scarlet fever and diphtheria.

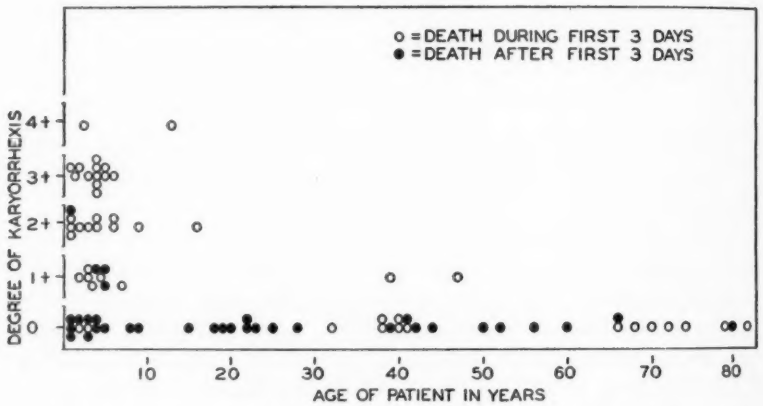
Observations

In the observations which I have made I have concentrated on a single, easily recognized feature of the changes described in the secondary centers (germinal centers) of splenic lymph nodules, or, more accurately, lymph cords. This feature is karyorrhexis (nuclear fragmentation). Usually when much karyorrhexis was present the secondary centers were large. The degree of karyorrhexis is expressed from 0 to 4 plus, varying from no observable karyorrhexis to the highest amount noted. If the degree of karyorrhexis so observed is correlated with the age of the patient (Text-Fig. 1) it is obvious that high degrees of karyorrhexis are noted only in the young and almost exclusively in persons less than 17 years of age. More than a 1 plus degree of karyorrhexis was not encountered in a single subject beyond adolescence.

In Text-Figure 1 the cases are divided into two groups depending on

whether the survival period was more or less than 3 days. It is noted that in only a single case with death after the third day was the degree of karyorrhexis as much as 2 plus. Thus the young (*i.e.*, those less than 17 years old) who died more than 3 days after receiving a burn failed to show the high degree of karyorrhexis which was exhibited by the young who died within 3 days.

The significance of these clear-cut results is difficult to evaluate, but several aspects may be discussed. It might be concluded that the burn is the cause of the high degree of karyorrhexis in the young. However, it may be that this degree of karyorrhexis was already present in the



Text-Fig. 1. Correlation of the age of the burned patient, duration of survival following burning, and the degree of karyorrhexis in the germinal centers of the splenic nodules. Each dot or circle represents a case. High degrees of karyorrhexis in the splenic nodules in fatal cases of burns occurred only in the young, *i.e.*, in those less than 17 years of age. In addition, high degrees of karyorrhexis occurred only in those who died during the first 3 days following the burn, regardless of age.

young, and that the burn which killed the child merely permitted the pathologist to see something normally present in the spleen. If this were so, it was also true that the condition present "normally," and during the first 2 days after the burn, disappeared some days later, possibly due to inanition, infection, or some other factor.

To try to determine the normal degree of karyorrhexis in the splenic nodules, sections of spleens were obtained from persons who had died within 24 hours after accidental injury such as an automobile accident, shooting, stabbing, strangulation, or suffocation. In many cases death was instantaneous. Only subjects at least 1 year of age were used.

In several thousand autopsies, 54 cases of such violent deaths were found, but unfortunately only 2 of these cases were young children

(5 and 3 years of age). These showed 1 plus karyorrhexis. Two plus karyorrhexis was shown by 2 adults, aged 21 and 45 years. None showed 3 plus or 4 plus karyorrhexis. Thirty-nine showed no karyorrhexis and among these there were no children less than 10 years of age. Hence one obtained the impression that normally the splenic nodules do not exhibit a high degree of karyorrhexis, but obviously the number of children in the series is too small for a conclusion. It would be desirable to examine a large series of accidental deaths in children, with death occurring soon after the accident, in an institution where such autopsies are available, in order to determine the degree of karyorrhexis present normally.

Karyorrhexis was observed within a few hours after a burn. Karyorrhexis of 3 plus degree was noted in a child who survived only 3 hours, and karyorrhexis of 4 plus degree was noted in a child who survived only 6 hours. Thus if the burn is the cause of the nuclear fragmentation the lesions develop with great speed. It may be that nucleated cells of the blood or of the tissues are damaged by the burn, just as red cells are,[20] and that particles of the injured nuclei are brought to the splenic nodules and deposited there. Or it may be that the cells of the lymph nodules are damaged by a toxic substance brought to them in the blood. Attempts to determine the origin of the nuclear particles by microscopic study were not successful. Some of the particles suggest the lobes of necrotic polymorphonuclear cells while others suggest pyknotic lymphocytes. Usually the particles are within the cytoplasm of macrophages. Occasionally the macrophages of the secondary centers of the splenic nodules also contain portions of red blood cells.

The failure to find karyorrhexis of high degree more than 3 days after receipt of the burn may indicate that sufficient time has elapsed to permit the macrophages to digest the nuclear material, or at least to destroy its property of absorbing the basic stain. This is in accord with the interpretation of Weiskotten.[6]

Kidneys

Sections of kidney from 88 of the cases were examined. A small number, only 9 cases, showed definite recent lesions. Infections, resulting from the burns, were present in the cases with renal lesions in which death occurred in the second week or later. These infections constituted a thoroughly satisfactory cause for the lesions noted. If these cases were excluded, only 4 remained in which renal damage was present and might have been a direct effect of the burn. In 1 of these 4 cases in which death took place 12 hours after the burn there were

hyaline droplets in the tubular epithelium, but diabetes was a complicating factor. In the 3 cases with death on the third day the renal changes might well have been due to the burn itself, but in all 3 cases there were complicating factors also, which might have been responsible for the changes observed. The type of damage and attendant circumstances in these 3 cases with death on the third day are given in the three succeeding paragraphs.

In the first case there was necrosis of the renal tubular epithelium of a moderate grade, with no obstruction of the collecting tubules. A third degree burn of four-fifths of the body surface had been sustained, hematuria had been present, and sulfadiazine spray had been used locally and plasma intravenously.

In the second case there was patchy necrosis of renal tubular epithelium. Epithelial cells were dislodged and had been caught in the tubules farther down. Hyaline droplets were present occasionally in the tubular epithelial cells. Extensive burns had been sustained and lobular pneumonia had developed.

In the third case there was minimal dilatation of the convoluted tubules. There was also focal necrosis of liver and hemorrhagic ileitis and colitis in this subject of 68 years.

No such spectacular lesion as that described by Brown and Crane,[21] bilateral cortical necrosis, was encountered in any of the cases in the entire series.

The cases at Johns Hopkins Hospital were examined before I knew about the importance of hemoglobinemia and hemoglobinuria in burns,[20] and I probably did not examine with sufficient care the contents of the tubules for the presence of hemoglobin casts. The lesion in the distal convoluted tubule and elsewhere in the kidney which has been described recently for the crush syndrome [22] and also in patients with burns [23] may have been overlooked.

The cases at Duke Hospital were re-examined for hemoglobin casts and changes in the distal convoluted tubules. Seven cases with patients surviving a short time were available, death occurring after 3, 12, 14, 22, and 40 hours, and 4 and 8 days, respectively. In 2 cases there were appearances suggesting hemoglobinemia, though this had not been established clinically.

The first of these was a woman, 39 years of age, who had received deep charred burns over 60 per cent of the body surface. Plasma (2300 cc.), whole blood (100 cc.), and cortin had been given. Death occurred 14 hours after the burn was received. At autopsy the bladder was empty. Blister fluid was pink as was much of the subcutaneous edema fluid. The intima of the aorta was stained intensely red. In the

blood vessels of the kidney distorted, shrunken, or conglomerated red cells occurred. The shrunken forms were identical to those described in the peripheral blood by Shen, Ham, and Fleming.[20] In the capsular spaces of the glomeruli eosin-staining material occurred which suggested hemoglobin. In an occasional collecting tubule there was eosin-staining fluid, débris, and rounded masses suggestive of hemoglobin.

In the second case, a child, death occurred at the end of 4 days. Burns were extensive and severe and had been treated with dry dressings. Oliguria and azotemia developed. In the distal convoluted tubules granular débris and masses with the eosin-staining qualities of hemoglobin were present, but there was no necrosis of the epithelium of the tubules. In a very rare collecting tubule débris which looked like hemoglobin was present.

In summary, even with these added considerations, renal changes in the series of burns were not impressive, and changes noted in patients dying in the second week or later were explained best on the basis of the secondary infection and septicemia which was usually present. In 3 cases, with death on the third day, it is possible that the changes were the direct result of the burns, but even in these cases there were complicating factors. Hemoglobin casts would undoubtedly have been found in some of the Hopkins cases upon re-examination.

Adrenal Glands

According to Weiskotten [6] the most prominent and characteristic of the necropsy findings in patients with burns are the changes in the adrenals. He described swelling, redness, and periadrenal edema with hemorrhage in all cases of more than 24 hours' duration. The weight of the adrenals in one of his cases was three times normal. Microscopically there were congestion and hemorrhage, and the gland cells were pale-staining and much swollen. Necrotic gland cells being invaded by polymorphonuclear and large mononuclear cells were not infrequent.

Observations

In the majority of cases the adrenal gland did not show arresting changes grossly, and the organ was usually described as normal, or congested, or as showing periadrenal edema.

I examined sections of the adrenal glands stained with hematoxylin and eosin from 68 cases. An analysis of the microscopic observations indicated the following: (1) Most of the cases showed no impressive change in the adrenal; (2) Congestion in and about the adrenal, periadrenal edema, and rarely periadrenal hemorrhage (not massive hemorrhage) were noted in several cases, especially during the first 3

days; (3) Necrosis was noted in 2 cases and was due to infection. It was thought that the congestion, periadrenal edema, and occasional periadrenal hemorrhage were probably features of shock and of the shifts of fluid which occur in burns. It is to be borne in mind, however, that changes in the adrenal of a functional metabolic nature could not be evaluated with any degree of refinement by simply looking at a section of adrenal gland stained with hematoxylin and eosin. Study of fat stains, granule stains, and quantitative methods might reveal changes.

Since the observations in the preceding paragraphs were made I have noted two pertinent references concerning adrenal lesions. Mallory and Brickley [24] reported focal necrosis of the adrenal in 2 cases in which death followed the Cocoanut Grove fire by 2 days in one instance and by 3 days in the other. They mentioned splitting of the cords of the outer portion of the cortex with accumulation of serous exudate in the space produced, pyknotic nuclei, acidophilic necrosis of adrenal cells, and infiltration of polymorphonuclear cells. Rich [25] has recently described a peculiar type of adrenal cortical damage associated with acute infections and has discussed the possible relation of this damage to circulatory collapse. The lesion consists of necrosis of isolated cells and a striking transformation of the solid cords of the zona fasciculata into tubular structures containing an inflammatory exudate.

In view of these observations, sections of adrenal were re-examined in cases autopsied at Duke Hospital with death occurring 3, 12, 14, and 40 hours, and 8 and 20 days following burns. In all of these cases, except in that with death on the 20th day, shrunken, dark-staining, apparently pyknotic nuclei were found occasionally in the cells of the cords of the zona fasciculata; and in 1 case, with death at 40 hours, some cells of the zona fasciculata had intensely eosin-staining cytoplasm in addition to pyknotic nuclei. Accumulations of inflammatory cells were not seen about the cells with intensely staining nuclei. I am unable to say whether actual necrosis was present or whether vagaries in staining accounted for the appearances observed. In the case in which death occurred 3 hours after the burn there was slight separation of the cells of cords of the zona fasciculata to form spaces, but this did not approach the tubule formation described by Rich [25] as characteristic of his cases with infections of various sorts.

Comment

Other organs than those previously mentioned showed changes but these were not subjected to analysis. Congestion and hemorrhage were frequently observed in the earlier deaths. Petechial hemorrhages of the epicardium and endocardium, lungs, stomach and duodenum, and else-

where were noted, as were occasional Curling's ulcers. Extensive hemorrhages into the lungs, with infarct-like areas, occurred in some cases. Congestion of many viscera, such as the spleen and liver, was noted in the cases in which death occurred after a short period of survival. The changes in the lymphoid nodules of the lymph nodes and gastrointestinal tract paralleled the changes in the splenic nodules. No definite or constant alteration was found in the brain. Mention should be made of necrosis and inflammation in the respiratory tract, which occurred from the direct inhalation of flames and fumes.

In the literature on burns, mention is made of the direct effect of heat on the internal organs,[26] with, for example, the production of large vacuoles or bubbles in the liver. Fat embolism has been reported.[10]

Nevertheless, the emphasis should be on the paucity of lesions in the internal organs following fatal burns. If the early changes are interpreted as those of shock and the slightly later ones as those of hemoconcentration, and if the changes in the blood cells and the hemoglobinemia in deep burns are recognized, there are possibly no additional morphologic alterations characteristic of burns other than the changes at the site of the burn. The liver usually shows no necrosis, the changes in the adrenals are possibly those of shock, and karyorrhexis in the lymphoid nodules occurs only in children. There is thus little support for the concept of a powerful burn toxin on the basis of pathologic studies.

Summary and Conclusions

Available for analysis was a series of 96 autopsies in which cutaneous burns or the complications of cutaneous burns were the chief cause of death. The series included 37 cases with death during the first day, 26 cases with death from the second to sixth day inclusive, and 33 cases with death after the first week.

Hepatic necrosis was usually absent, and when present could be explained more reasonably as a result of a complication of the burn, such as infection, then as a direct result of the burn. Necrosis of minimal degree was noted in 4 cases in which no factor but the burn was demonstrated as a cause. In this series of cases tannic acid treatment had not been used to any appreciable extent. In 28 cases from the period before the use of tannic acid there was no case of hepatic damage due to the direct effect of the burns. It was concluded that necrosis of the liver is not a lesion characteristic of burns.

Karyorrhexis of high degree occurred frequently in the lymph nodules of the spleen in those patients less than 17 years of age who died during the first 3 days following burning. Karyorrhexis was absent, or present in minimal degree, in the splenic nodules of those older

than 17 years. Moreover, when those less than 17 years of age survived more than 3 days, karyorrhexis was absent or present in minimal degree. While it could not be proved that the striking karyorrhexis present in the young who died during the first 3 days was not present before burning, it was thought that the karyorrhexis was probably the result of the burn and that it disappeared after the third day because of the digestion of the nuclear particles by phagocytic cells rendering them nonstainable with hematoxylin. It was concluded that changes in the splenic nodules were not fully characteristic lesions of fatal burns, since these changes were not present in adults.

Unequivocal changes in the adrenal and kidney were infrequent. The swelling, congestion, and occasional homorrhage in the adrenals in early deaths were attributed to shock. Hemoglobin casts were noted in the kidneys rarely.

In general, emphasis is placed upon the paucity of histopathologic alterations specific for burns and not attributable to shock, to the rarely occurring hemoglobinemia, or to secondary infection.

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[*Illustrations follow*]

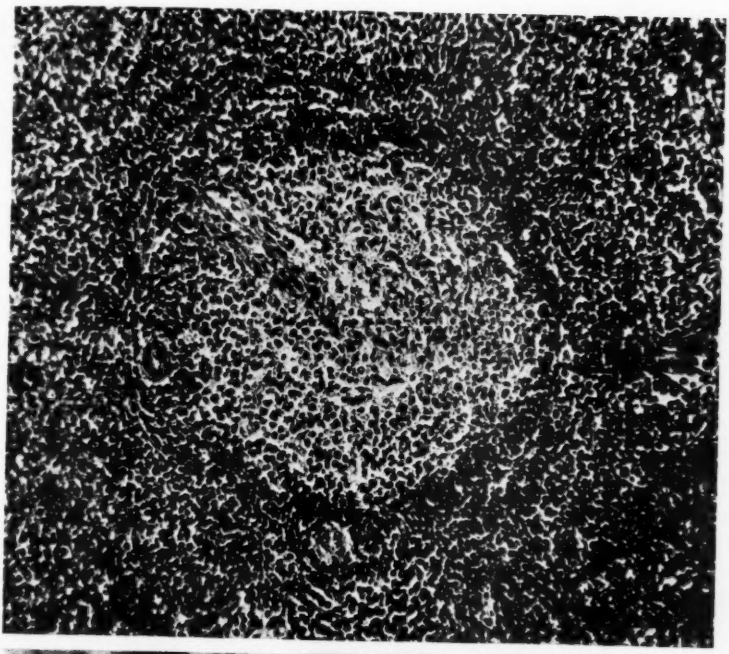
DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Low-power photomicrograph of a splenic nodule from a child, 4 years of age, who survived 3 hours following a burn. The nodule has a large secondary center (pale) with a rim of lymphocytes about it. Hematoxylin and eosin stain. × 50.

FIG. 2. Oil-immersion field from the center of a secondary nodule of the same case as in Figure 1, showing karyorrhexis of 3 plus degree. Most of the nuclear fragments are within phagocytic cells. This case demonstrates that high degrees of karyorrhexis may be encountered as soon as 3 hours after a burn in those less than 17 years of age. Hematoxylin and eosin stain. × 1,458.

1



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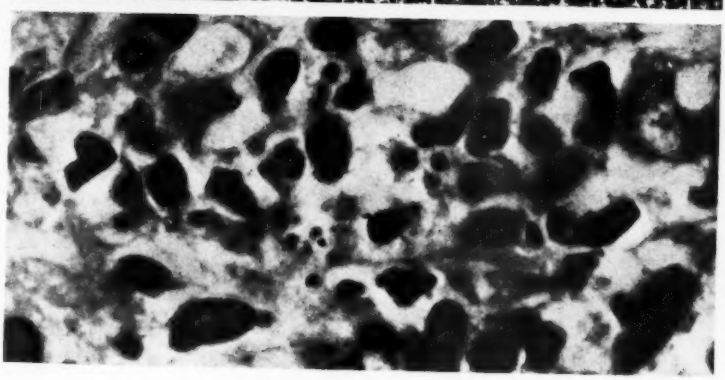


Baker

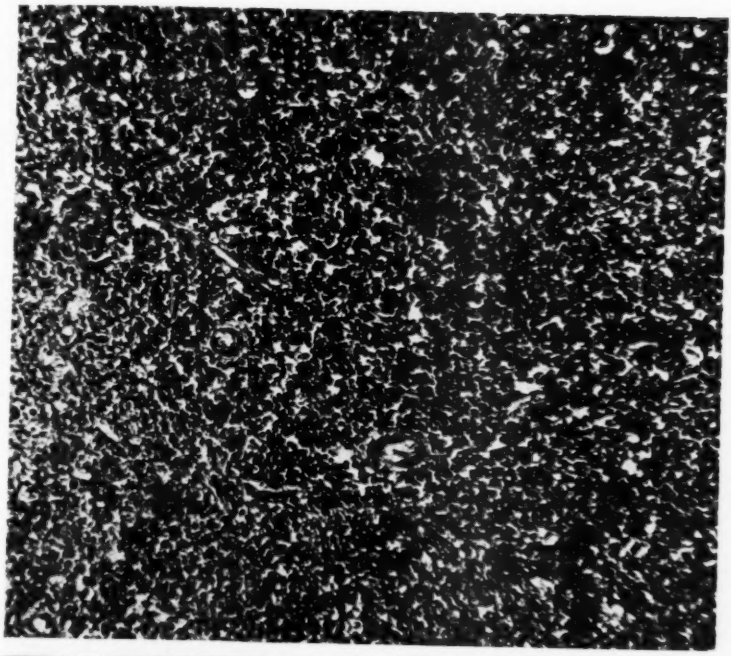
Internal Lesions in Burns

PLATE 123

FIG. 3. Low-power photomicrograph of a splenic nodule from a child, 3 years of age, who survived 20 days following a burn. The nodule contains no secondary center, and typifies the nodules throughout the section. Hematoxylin and eosin stain. × 50.

FIG. 4. Oil-immersion field from the center of the splenic nodule of the same case as in Figure 3. No karyorrhexis is present. This case conforms with the general experience that high degrees of karyorrhexis were not encountered in those patients less than 17 years of age if they survived more than 3 days following the burn. Hematoxylin and eosin stain. × 1,458.

3



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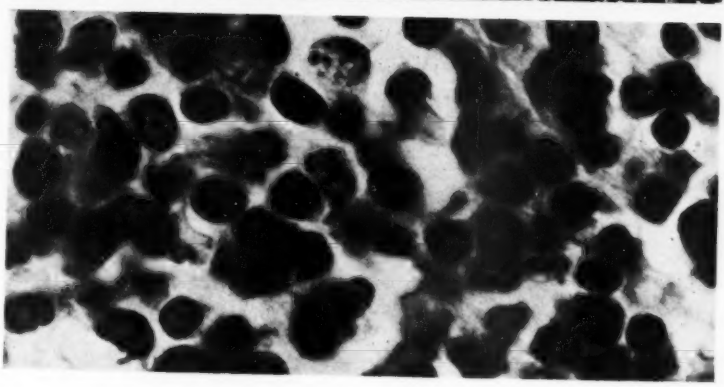

Baker Internal Lesions in Burns

PLATE 124

FIG. 5. Mid-portion of a secondary center of a splenic nodule of a child, 6 years of age, who survived 22 hours following a burn. Karyorrhexis of 3 plus degree is present. As in this example, marked karyorrhexis is usual in young persons dying during the first 3 days following a burn. Hematoxylin and eosin stain. × 1,458.

FIG. 6. Center of a splenic nodule and edge of a secondary center from a child of 5 years who died 8 days following a burn. Karyorrhexis of 1 plus degree is present. As shown in this case, high degrees of karyorrhexis are not noted in young persons who survive more than 3 days following a burn. The photomicrograph also shows cells with abundant hyaline cytoplasm which may represent material previously in the form of nuclear particles, but which now fails to stain because of intracellular digestion. Hematoxylin and eosin stain. × 1,458.

FIG. 7. Center of a splenic nodule from an adult, 38 years old, who survived 12 hours following a burn. Karyorrhexis is absent. This conforms with the observation that high degrees of karyorrhexis were not observed in adults. Hematoxylin and eosin stain. × 1,458.

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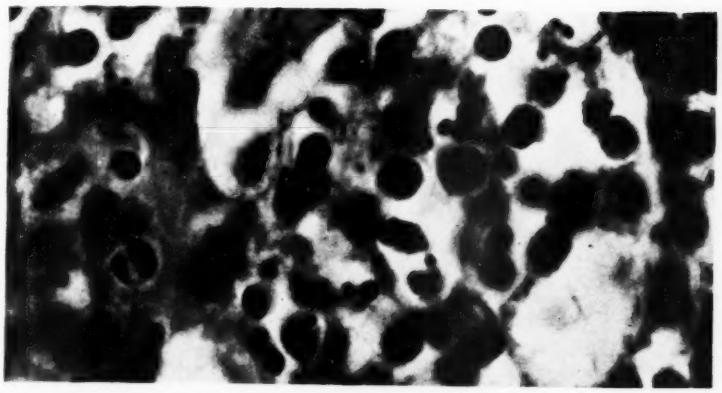

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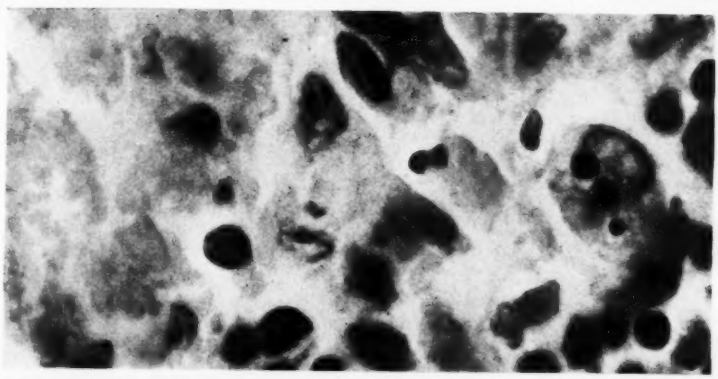

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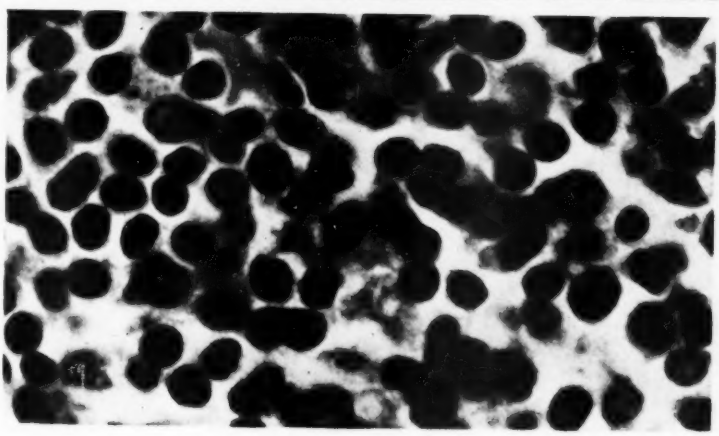

THE "MASSON BODY" IN RHEUMATIC PNEUMONIA *

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Although the morphologic features of rheumatic fever as it affects the heart, serosal surfaces, and arterioles are well known and agreed upon, the same cannot be said of its manifestations in the lungs. While most observers believe that there is a rheumatic pneumonia, their opinions vary with regard to the exact nature of the lesion. There are (1) those who believe the changes in the lungs are specific,[1-4] (2) those who maintain that they are characteristic but not specific,[5] and (3) those who contend that they are neither characteristic nor specific.[6-8] For the early or acute stages there have been described acute necrotizing and fibrinous alveolitis; hemorrhages; hyaline membranes at the periphery of the alveolar spaces; infiltration of the septa and spaces with monocytes, pigment cells, neutrophils, basophils, plasma cells, and lymphocytes; acute periarteritis with intimal proliferation and hyaline thrombi in the lumina and even Aschoff nodules similar to those found in the myocardium. Later, varying stages of organization and fibrosis have been reported.

Apart from the above-mentioned changes, Masson, Riopelle, and Martin,[9] in 1937, described an inflammatory reaction in the lungs of 13 cases consisting of a peculiar type of cellular granulation tissue in the form of buds which filled the alveolar ducts and spaces. They concluded that rheumatic pulmonary involvement was a specific process. In 1944, Neubuerger, Geever, and Rutledge [10] reported on 8 cases, including both active and quiescent rheumatic fever, in which they found similar granulomatous nodules. They described them as consisting of a central core of pleomorphic cells, some possessing flat spindle-shaped nuclei with scanty neutrophilic cytoplasm and others round, oval, or kidney-shaped nuclei with abundant sharply demarcated cytoplasm. Some of the cells contained golden-brown pigment. The supporting stroma of fibroblastic tissue was loose and fibrillary and contained fibrinous and sometimes mucoid material. Occasionally fibrinous material was collected into a band at the periphery of the granuloma. Capillaries were scanty or entirely absent. Some of the granulomas were covered externally with a single layer of cuboidal cells of "septal" origin. These peculiar formations they termed "Masson bodies" and since similar nodules were not found in 60 control cases of acute pneumonia, passive congestion of the lungs with and without pneumonia, and chronic organizing pneumonia, they concluded that they were

* Received for publication, August 5, 1944.

specific for the rheumatic lung and that they were equivalent to the Aschoff body in the heart.

It is the purpose of this presentation to consider solely the question of specificity of the "Masson body" in rheumatic pneumonitis and to omit entirely a discussion of the already often described and still controversial changes previously referred to.

Method and Material

Microscopic sections of the lungs of 505 cases were studied. They were distributed as follows: uncomplicated rheumatic heart disease, 150; bronchiectasis, 160; tuberculosis, 85; chronic interstitial pneumonitis, 40; organizing pneumonia, 30; abscess, 25; asthma, 12; and periarteritis nodosa, 3. With the exception of 120 surgically removed bronchiectatic lungs, all were obtained from the necropsy file of the Jefferson Medical College Hospital, covering a period of 15 years. Rheumatic heart disease was excluded in the control series by a direct examination of the heart in the cases autopsied and by a careful examination of the histories of the patients whose surgically removed lungs showed the granulomatous bodies. Only those cases were included for which there was at least one satisfactory slide of the lung, and, in the instances of rheumatic heart disease, where there was in addition at least one satisfactory section of the heart. For the most part only file slides were examined. In some cases the original blocks were recut and in a few, where gross specimens were still available, additional material was obtained for study. All sections were stained with hematoxylin and eosin.

Results

Rheumatic Heart Disease

In the 150 cases of rheumatic heart disease, Aschoff nodules were present in the myocardium in 16 and absent in 134. "Masson bodies" were found in the lungs in 3 cases of each group.

Grossly, the lungs of these 6 cases disclosed no characteristic or even constant changes. In 1 case the pleural surfaces were covered with a fibrinous exudate which on closer inspection showed numerous, small, irregularly distributed elevations measuring 1 mm. in height and 3 to 4 mm. in diameter. Two common findings in the lungs proper were an increased resistance upon palpation and, on section, varied degrees of congestion and edema. Irregular areas of pneumonic infiltration were also observed, but they did not differ in any way from those of ordinary pneumonia.

Microscopically, the outstanding feature was the patchy distribution of the "Masson bodies." Their histologic structure varied somewhat but the general pattern appeared to be the same in all cases. The nodules might conveniently be divided into two groups, early and late, although it is to be understood that there was no sharp line between the two, but rather a gradation from one to the other. The early lesions seemed to arise either in the septa proper or within the alveolar ducts or spaces. Those of the former origin were by far the less frequent. Each consisted of a round or fusiform, unilateral or bilateral bulging of the septum caused at first by a fibrinoid necrosis of the wall and later by a gradual replacement with loose fibrillary connective tissue and a mixture of various types of inflammatory cells. As the process progressed the nodules became more circumscribed but still retained their septal connections. Sometimes it was impossible to tell whether the lesion started in the septum or without. Ordinarily, however, the latter origin was quite evident and constituted the more frequent site.

In 2 patients who were 3½ and 7 years old, and who apparently died in the initial attack, the lesions in the alveolar spaces and ducts were very early and in various stages of development. They consisted essentially of a mixture of fibrin, monocytes, and very occasionally polymorphonuclear leukocytes, plasma cells, and pigment cells. Usually fibrin predominated. While some of these arrangements were unattached to the alveolar septa, most of them were united to the latter by a broad base or one or more slender pedicles. As the lesions progressed the fibrin became less deeply stained and was gradually replaced with loose edematous connective tissue. Externally many of the bodies were partially or almost completely covered with a single layer of flat or low cuboidal cells. Occasionally there was a condensation of the fibrinous and fibroblastic material at the periphery of the nodule while the center was filled with monocytes, pigment cells, and a few plasma cells.

The distribution of the nodules in the late stage, as in the early, was also patchy. In all sections where "Masson bodies" were found, however, there were also present a few irregular plugs of organizing or organized exudate. These were essentially similar in structure and age to the "Masson bodies," and differed only in their external configuration. Again, as in the early lesion, most of the granulomas were attached to the ductal and septal walls at one or more points by narrow or broad pedicles. Sometimes, however, they appeared to be a direct expansion of the septum or alveolar duct wall itself. They were all sharply circumscribed and round, oval, or less frequently fusiform. Often there was an external covering of a single row of flat or cuboidal cells. Most

of them contained neither blood vessels nor erythrocytes. They were easily divided into four types, namely: (1) fibrillary, consisting of fibrin mixed with edematous fibroblasts and often arranged more or less concentrically like the layers of an onion (Fig. 1); (2) fibrous, consisting entirely of dense fibrous tissue (Fig. 2); (3) granulomatous, consisting of a few spindle cells with spindle-shaped nuclei; large, round or oblong cells with abundant pink-staining cytoplasm and large, round, evenly stained nuclei; phagocytes containing ingested brown pigment, and small round cells with almost imperceptible cytoplasm (Fig. 3); (4) mixed forms of the above, consisting of the various elements just mentioned, either irregularly scattered throughout the nodules, or separated into a central core of inflammatory cells surrounded peripherally by a band of fibrinous material or fibrous tissue. Ordinarily all types of granulomas were found in the same patient and even in the same slide.

Bronchiectasis

In 160 cases of bronchiectasis there were 26 that showed "Masson bodies." These were in all respects similar to and frequently identical with, those found in rheumatic lungs. Their distribution was patchy and only in a few instances were there more than two or three in a single field (Fig. 4). Some appeared to have originated in the septa while others seemed to have started in the spaces and to have become attached to the adjoining walls by broad or thin pedicles. Structurally, as in the rheumatic lungs, they were either (1) fibrillary (Fig. 5), (2) fibrous (Fig. 6), (3) granulomatous (Fig. 7), or (4) mixed. The granulomatous type differed slightly from those similarly designated in the rheumatic lungs. Cellularity was usually more marked; plasma cells were more abundant; phagocytic cells contained ingested lipoid material instead of pigment, and, finally, the surrounding alveolar septa were broader and densely infiltrated with a mixture of similar cells. The remaining three types of bodies in this group were so similar to those in the rheumatic lungs that in some instances they could practically be superimposed. Organized exudate similar in composition to the "Masson bodies" was also seen scattered throughout.

Tuberculosis

In 85 cases of pulmonary tuberculosis there were 7 that showed "Masson bodies." As in the rheumatic and bronchiectatic lungs, they included all types previously referred to. Some were almost mirror images of those found in bronchiectasis and rheumatic heart disease (Fig. 8). All cases that showed "Masson bodies" also showed plugs of exudate in varying stages of organization.

Chronic Interstitial Pneumonitis

In 40 cases of chronic interstitial pneumonitis there was only 1 case that showed a few "Masson bodies" of the granulomatous type (Fig. 9). Here, however, as in the granulomatous type seen in bronchiectasis, the structure was somewhat different from that of the third type seen in the rheumatic lungs. The cells were mostly plasma cells and the septa from which they sprang were greatly thickened as a result of infiltration with similar cells. None of these 40 cases showed organizing pneumonia.

Organizing Pneumonia

In 30 cases of organizing pneumonia there were 14 with "Masson bodies." In all of these the pneumatic exudate was in various stages of organization, and was the most conspicuous change. There were, however, two other outstanding features in this group of cases. Firstly, the bodies were practically all of the fibrillary or fibrous types; and, secondly, capillaries and erythrocytes were practically non-existent. In some cases there was unmistakable evidence that the organized exudate in the spaces became attached to the alveolar wall (Fig. 10). In others the nodules were already rounded off and covered with cuboidal or attenuated cells. Golden-brown or black pigment was frequently seen within many of the bodies of the fibrous type.

Pulmonary Abscess

In 25 cases with pulmonary abscesses there were 5 that showed "Masson bodies." These were of the granulomatous, fibrous, and mixed types. Several of the mixed type had a broad peripheral band of fibrous tissue which also formed the main part of the pedicle (Fig. 11). The central portion was filled with spindle cells, plasma cells, small round cells, and phagocytes with and without pigment. There were no capillaries. All cases showing "Masson bodies" also disclosed alveolar exudate in similar stages of organization.

Asthma

Of 12 cases of asthma none showed "Masson bodies" and none showed organizing or organized pneumonia.

Periarteritis Nodosa

In 3 cases of periarteritis nodosa there was 1 that showed a "Masson body." It appeared to be a bilateral bulging of a septum (Fig. 12). It was composed of loose edematous and fibrillary connective tissue and contained scattered plasma cells and pigment cells. One surface was covered with low cuboidal cells. Nearby there was an organizing infarct

and the neighboring alveolar spaces contained scattered plugs of organizing exudate.

Discussion

Although Neubuerger, Geever, and Rutledge [10] "discovered the 'Masson body' in a few instances wherein no previous rheumatic history was elicited," they nevertheless concluded that despite a few exceptions of this type "the 'Masson body' is a fairly specific granuloma." In a larger and somewhat different series of control material we have found the "Masson body" in a high proportion of cases of organizing pneumonia, pulmonary abscess, bronchiectasis, and tuberculosis, and are therefore of the opinion that such granulomas are not specific for rheumatic pneumonia but are found in a wide variety of pulmonary disorders. That the control cases were not also affected with rheumatic fever was made reasonably certain in the autopsied group by a direct examination of the heart, and in the surgically removed lungs, showing "Masson bodies," by a careful examination of the case histories. Since most of the positive cases were obtained by an examination of consecutive file slides, with frequently only one slide available from a case, we are also certain that had more sections from different areas of the lungs been examined, the number of positives would have been much higher.

The relation of the "Masson body" to organizing or organized pneumonia is of more than passing interest. Although Neubuerger, Geever, and Rutledge [10] gave special attention to the differential diagnosis between the two, some of the differences mentioned were not too apparent in our material. It is noteworthy that all cases in this series, both rheumatic and non-rheumatic, that showed "Masson bodies" also disclosed in the same sections a few scattered plugs of unmistakable organized pneumonic exudate. Furthermore, the structural composition of both the "Masson body" and the organized exudate was generally the same. When the former was composed of fibrillary connective tissue so was the latter. When one possessed a few pigment or foam cells so did the other. When there was an external covering of cuboidal cells on the "Masson body," so were there similar cells covering the organized exudate. Capillaries and extravasated erythrocytes were practically nonexistent in either structure. For these reasons we believe that in most cases the "Masson body" is organized nonspecific exudate which has assumed a polypoid, round, or oval shape and become attached to the septal or ductal wall as the inevitable result of organization. In fact, in the acute rheumatic pneumonia described in this report, all graduations from the presence of a mass of fibrinous material in the alveoli and ducts to the fully formed "Masson body" have been traced.

On the other hand it should again be pointed out that occasionally these granulomas do originate in the septal wall itself. This was seen definitely in some of the cases of rheumatic fever, in bronchiectasis, and in chronic interstitial pneumonia.

The cases of asthma and periarteritis nodosa were included in this report because it was thought that if the "Masson body" is specific for rheumatic fever, which is probably an allergic disease, it should also be found in asthma and periarteritis nodosa. Unfortunately the number of cases at our disposal was too small from which to draw definite conclusions. It might, however, be of interest to note that, although many histologic sections of the lungs of these cases were examined, only one "Masson body" was found in one case of periarteritis nodosa, and nearby there were an organizing infarct and a few irregular plugs of organized pneumonic exudate.

Summary and Conclusions

In a study of the lungs of 505 cases, "Masson bodies" were found in approximately 46 per cent of those with organizing pneumonia; 20 per cent, with pulmonary abscess; 16 per cent, with bronchiectasis; 8 per cent, with pulmonary tuberculosis; 4 per cent, with rheumatic heart disease; 2 per cent, with chronic interstitial pneumonitis; 1 of 3 cases of periarteritis nodosa; and in none of 12 cases of asthma. In all these cases there were also a few nearby plugs of organized exudate which did not differ from the "Masson body" in their structural composition, but only in their external configuration. We are therefore of the opinion that most "Masson bodies" are organized intra-alveolar and intraductal exudate, and that only a few originate in the septal and ductal walls. Since these granulomas are found in a wide variety of pulmonary disorders, they are not specific for rheumatic pneumonitis.

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DESCRIPTION OF PLATES

PLATE 125

FIG. 1. From a case of rheumatic fever, showing a "Masson body" of the fibrillary type. It has two pedicles and is composed of fibrin and loose fibrillary connective tissue with very few central plasma cells. There is a single layer of flat cells covering a portion of the periphery. Hematoxylin and eosin stain. × 200.

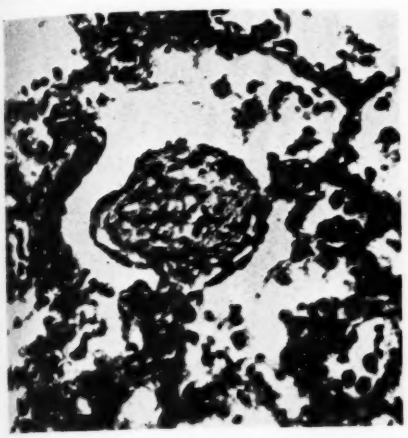
FIG. 2. From a case of rheumatic fever, showing a "Masson body" of the fibrous type attached to a septum at one point. It is composed of dense fibrous tissue and is covered with a single layer of flat cells. Hematoxylin and eosin stain. × 200.

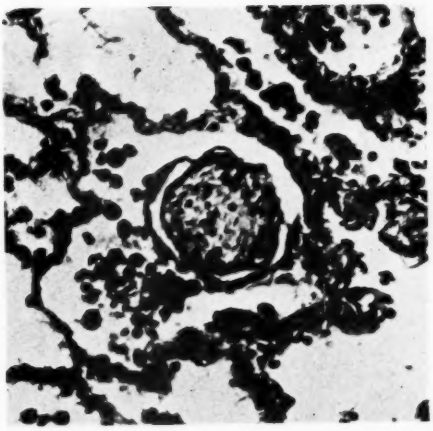
FIG. 3. From a case of rheumatic fever, showing a "Masson body" of the granulomatous type. It has a broad base and is composed of spindle cells, round cells with abundant and distinct cytoplasm, round cells with almost imperceptible cytoplasm, and phagocytes containing brown pigment. There are a few peripheral cuboidal cells at the tip. Hematoxylin and eosin stain. × 200.

FIG. 4. From a case of bronchiectasis, showing seven "Masson bodies" of the fibrillary type. Hematoxylin and eosin stain. × 50.

FIG. 5. One of the bodies in Figure 4 at a higher magnification. It is composed of loose fibrillary connective tissue somewhat concentrically arranged. The periphery is partially covered with a single layer of flat cells. Hematoxylin and eosin stain. × 200.

FIG. 6. From a case of bronchiectasis, showing a "Masson body" of the fibrous type. It is attached by a thin and broad pedicle and is composed of dense fibrous tissue. It has an incomplete external covering of flat cells. Hematoxylin and eosin stain. × 200.





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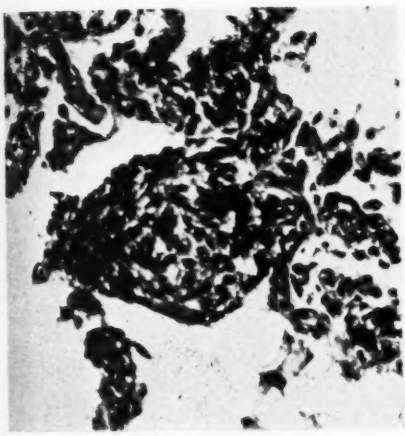

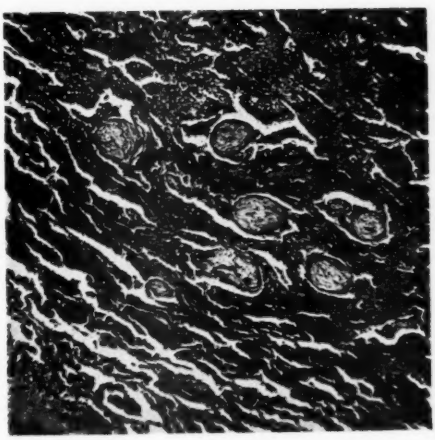

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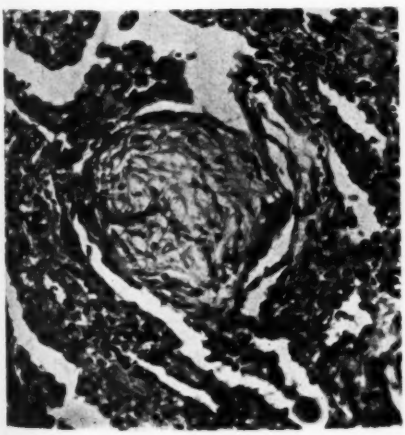

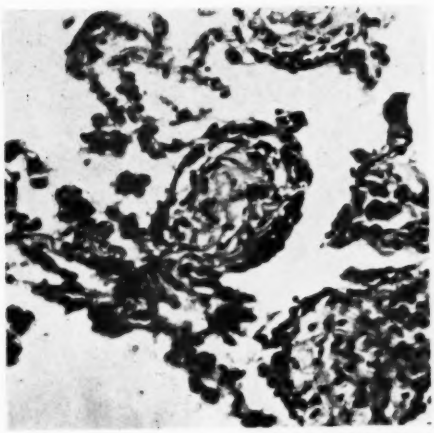

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PLATE 126

FIG. 7. From a case of bronchiectasis, showing a "Masson body" of the granulomatous type. It is attached by a thin pedicle and is composed of plasma cells, lymphocytes, a few polymorphonuclear leukocytes, many phagocytes containing ingested lipoid material, and two tiny capillaries. Externally there is a single row of cuboidal cells. Hematoxylin and eosin stain. × 200.

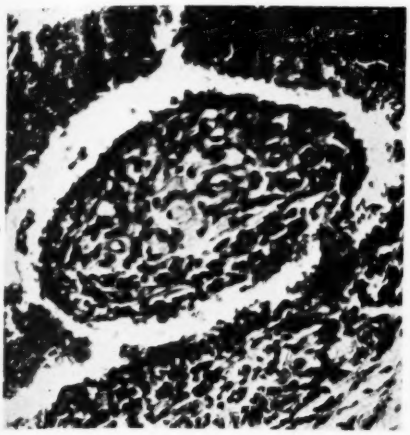
FIG. 8. From a case of pulmonary tuberculosis, showing a "Masson body" composed of concentrically arranged connective tissue and a central collection of plasma cells, lymphocytes, and phagocytes containing brown pigment. Hematoxylin and eosin stain. × 200.

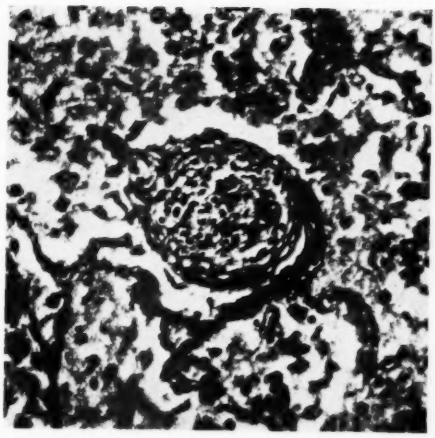
FIG. 9. From a case of chronic interstitial pneumonitis, showing a "Masson body" of the granulomatous type. It is attached by a thin pedicle and is composed almost entirely of plasma cells and fewer lymphocytes in a background of loose connective tissue. There is an external covering of a single layer of cuboidal cells. Hematoxylin and eosin stain. × 200.

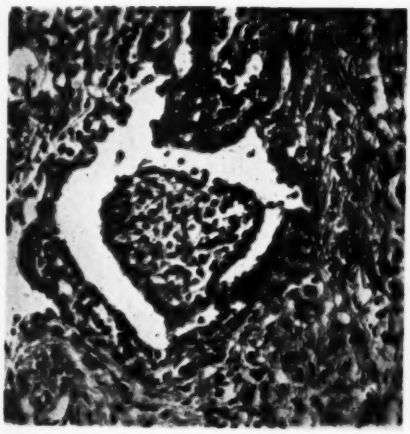
FIG. 10. From a case of organizing pneumonia, showing a transition stage between a plug of organized exudate and a "Masson body." It is composed of a mass of dense fibrous tissue united to the septa at two points. Centrally there are present a few plasma cells and phagocytes laden with brown pigment. Blood vessels are absent. Hematoxylin and eosin stain. × 200.

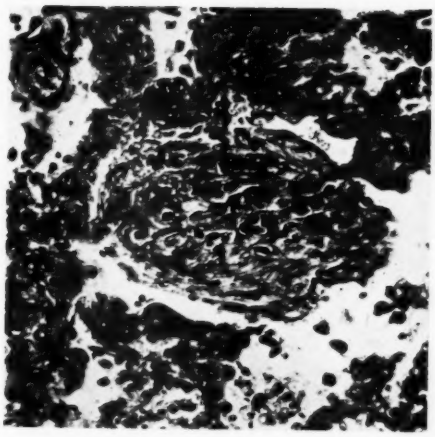
FIG. 11. From a case of pulmonary abscess, showing a "Masson body" of the mixed type. It consists of an outer zone and pedicle of dense fibrous and loose fibrillary connective tissue. Centrally there are spindle cells, plasma cells, lymphocytes, and phagocytes with and without brown pigment. Hematoxylin and eosin stain. × 200.

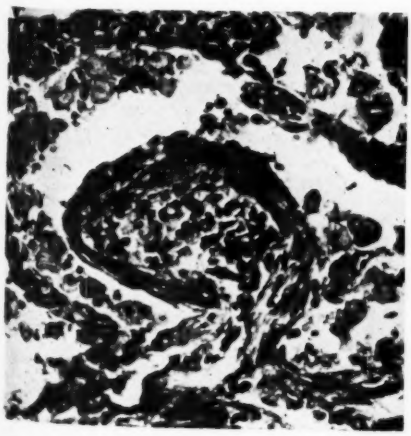
FIG. 12. From a case of periarteritis nodosa, showing a "Masson body" that seems to be arising as a bilateral swelling of a septum. It is composed of loose fibrillary connective tissue in which there are a few plasma cells and phagocytes containing brown pigment. One border is partially covered with a single layer of cuboidal cells. Hematoxylin and eosin stain. × 200.

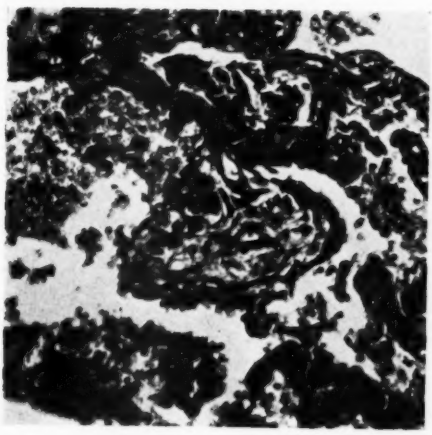












Herbut and Manges

The "Masson Body" in Rheumatic Pneumonia

THE REACTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL BRUCELLOSIS OF DOGS *

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The experimental studies reported in this paper relate to the general problem of the reaction of mammalian tissues to infection by *Brucella suis*, but with special emphasis upon the response of the cells belonging to the reticulo-endothelial system. This investigation was made as a part of a more comprehensive program, the main purpose of which is to test the possibility of an etiological relation between brucella infection in man and Hodgkin's disease. In previously published papers we have reported studies on experimental brucellosis in a variety of animals, including guinea-pigs,[1] hogs,[2, 3] dogs,[3] and on naturally acquired brucellosis in man,[4, 5] all of which relate to this problem. In all of the experimental studies the strains of brucella employed were derived from cases of typical Hodgkin's disease; a strain of brucella derived from a naturally infected hog was used for the production of comparative infection. In this paper are described in some detail the pathological anatomical findings in a group of dogs in which infection by a strain of *Br. suis* had been maintained for as long as 487 days by repeated inoculations. The bacteriological and immunological observations made in this experiment will be dealt with only in brief; they have been reported in full in a previous communication.[3]

The literature dealing with brucellosis in dogs, both the naturally acquired and the experimental disease, is quite limited; this has been reviewed in one of our earlier communications.[3] At this time only a brief note relating to the previously reported pathological anatomical findings is pertinent. It has been observed that the development of anatomical lesions in both the naturally acquired and the experimental infection is quite unusual; even so, in the hands of practically all experimenters, there appears to have been no great difficulty in establishing infection in the dog as indicated by a significant rise in the agglutination titer for brucella and the recovery of the organisms either from the tissues at autopsy or from the blood during life. The rare instances in which anatomical lesions have been described in brucellosis in dogs and the lesions described were: (a) enlargement and suppuration of the testis (Plantz and Huddleson,[6] Davis [7]), (b) multiple yellowish nodules in the kidney (Thomsen [8]), (c) enlargement of the reticulo-

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endothelial tissues, especially the lymph nodes and the spleen (van der Hoeden [9]), (d) focal, histiocytic, tubercle-like reactions in the liver, spleen, kidneys, and lungs (Feldman, Bollman, and Olson [10]).

Of the descriptions just mentioned, that of Feldman, Bollman, and Olson [10] is most informative. The lesions they describe were found in dogs experimentally infected with organisms obtained from swine and bovines. The dogs were inoculated by mouth and intravenously, only one inoculation being made. The total duration of the experiment was 185 days. The histological descriptions of the lesion by Feldman, Bollman, and Olson, unlike those of the other contributors, are quite comprehensive, and they indicate that the basic reaction in the dog is that which involves a response of the cells of the reticulo-endothelial system. In all of the studies that have been made, the conclusion has been reached that the dog has but little susceptibility to injury by brucella and that whatever lesions may be produced are minimal and transient. In view of this conclusion and in view of our primary objective, that is, to produce in the dog, if possible, a disease resembling Hodgkin's disease in man, it was decided to use, in our experiments, procedures that were significantly different from those utilized by preceding workers. Accordingly, in the experiments, reports of which follow, repeated inoculations were made by different routes and at considerable intervals of time, and the experiments were continued for as long as 300 days in excess of the experimental period in the investigations of Feldman, Bollman, and Olson.

Experiments

Nine healthy dogs were employed in our experiment. Seven of these were used for a study of infection by a strain of brucella obtained from a typical case of Hodgkin's disease in an adolescent boy who had died of the disease after an illness of over 5 years. The strain of brucella recovered from this case (herein referred to as the Brody strain) was of the suis variety. Previously it had been shown to be pathogenic for the guinea-pig, and it had been used in other experiments carried out on the hog; this organism was known to be virulent for the guinea-pig at the time the experiments on the dog were begun. Two of the dogs were used for a comparative study of infection by a strain of *Br. suis* which had been recovered from a natural infection in a hog (herein referred to as strain ABF 36). This organism was known to be pathogenic for the hog and the guinea-pig at the time the dog experiments were begun.

Preliminary studies involving the determination of the agglutination titer for brucella 456, the opsonocytophagic index, and cultures of the blood for brucella were carried out on all of the experimental animals.

Each animal was kept in its own cage from which it was never re-

moved except for inoculation. All animals were kept in a room in which there were also other animals infected by the organisms being employed in the experiment. Special care was exercised in the handling of all animals in this group to prevent cross infection.

Four of the animals inoculated with the Brody strain of brucella received these organisms intravenously. Three of the animals receiving the Brody strain were inoculated intraperitoneally. One of the animals receiving the ABF 36 strain of brucella was inoculated intraperitoneally

TABLE I

Experimental Brucellosis in Dogs: Experimental Data

Dog	Length of experiment in days	Organism and route	Injections	Blood cultures	Positive blood cultures	Days before death of:		Organ cultures at autopsy					
						Last injection	Last positive blood culture	Nodes	Spleen	Liver	Kidney	Testis	Lung
I	198 died	Brody I. V.	21	27	12	14	7	+	+	+	+	−	−
II	487 killed	Brody I. V.	35	42	18	225	233	+	−	−	+	−	o
III	461 killed	Brody I. P.	39	41	4	143	270	−	−	−	−	o	o
IV	261 died	Brody I. V.	28	34	23	7	7	o	+	+	+	+	−
V	38 died	Brody I. V.	4	5	3	8	1	+	+	o	o	o	o
VI	454 killed	Brody I. P.	39	43	o	136		−	−	−	−	−	o
VII	308 killed	Brody I. P.	33	34	8	132	231	−	−	−	−	o	−
IX	186 killed	ABF 36 I. P.	3	5	1	152	152	+	−	−	−	−	o
X	216 killed	ABF 36 I. V.	3	5	3	182	101	+	−	−	−	o	o

and the other intravenously. The inoculations were given repeatedly, usually at intervals of about 1 week. In certain instances the inoculations were given in two series, the second series being started after the animal had been allowed to recover sufficiently from the preceding inoculations to assure prolongation of the experiment.

All of the dogs were bled from the jugular vein at frequent intervals and always preceding inoculation. Blood cultures were made and brucella agglutination titers and opsonocytophagic indices were determined at each bleeding. The essential data relating to the experiment are recorded in Table I. The bacteriological and immunological observations made during the course of this experiment are the subject

of a previous communication in which all of the details may be found.[3] Protocols in summary for each of the experimental animals follow.

Dog I. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog I, a male mongrel, weighed 9.3 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 37; agglutination for brucella 456, negative. The animal died 198 days after the first injection.

Dog I received 21 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 14 injections were given at weekly intervals; from that time injections were given irregularly, at an average of bi-monthly intervals. The last injection was given 14 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.8° and 40° C. Opsonocytophagic index varied widely during the experiment. From an initial 37 it rose gradually to 91.5, then varied between 56 and 80.5. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week and varied from 1:2560 to 1:20480 during the experiment. Brucella was recovered from the blood stream after the first injection, and a total of 12 times in 27 weekly cultures. The positive results were obtained from cultures made 1 week after injections; blood cultures at intervals of 2 or more weeks following inoculations were always negative.

During the first 11 weeks of the experiment the dog appeared well. Then, with continued weekly injections he developed anorexia, rapidly lost weight, and at 14 weeks appeared moribund. At this time multiple shallow ulcers appeared in his mouth. These did not resemble the lesions of black tongue and did not show a characteristic response to adequate parenteral doses of nicotinic acid. Brucella was isolated from the ulcers 15 days after the last previous injection of organisms. When inoculations were discontinued the ulcers slowly healed and the general condition of the animal improved. When injections were started again and given at shorter intervals, the dog seemed to hold his own for a while, but then again developed anorexia, became very weak and cachectic, and at death presented a picture of extreme emaciation. No enlargement of the peripheral lymph nodes was observed during the experiment.

Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog II. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog II, a male mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 50; agglutination for brucella 456, negative. The animal was killed 487 days after the first injection.

Dog II received 35 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The inoculations were given at almost weekly intervals. The animal was then kept for the duration of the experiment without further injections. The last inoculation was given 225 days before death.

Temperature taken every 3 days during the first 2 months ranged between 38.4° and 39.8° C. Opsonocytophagic index varied widely during the experiment, from an initial 50 to a low of 35 and a high of 87. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week, and fluctuated from 1:1280 to 1:20480 during the experiment. Brucella was recovered from the blood stream

after the first inoculation, and a total of 18 times in 42 cultures. All of the positive cultures were obtained 1 week after inoculations; blood cultures at intervals of 2 or more weeks following injections were always negative.

During the first 30 weeks of the experiment the dog appeared well except for periods of anorexia and mild weight loss. He then developed signs of illness, including pronounced lassitude, anorexia, weakness, and conspicuous weight loss. After 37 weeks it appeared that the animal would not survive further injections, and the inoculations were discontinued. The dog gradually recovered and remained well for the rest of the experiment. No peripheral lymph node enlargement was noted during the period of observation.

Dog was killed by intracardiac air, and autopsy was performed immediately after death. Findings are summarized in Table II.

Dog III. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog III, a female mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 32; agglutination for brucella 456, negative. The animal was killed 461 days after the first injection.

Dog III received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These were given at weekly intervals except for the last few injections, which were given at bimonthly intervals. The last inoculation was given 143 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.9° and 39.1° C. Opsonocytophagic index fluctuated widely during the experiment. From an initial level of 32 it varied between 25.5 and 83. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and thereafter fluctuated between 1:1280 and 1:20480. Brucella was isolated from the blood 1 week after the first and the second inoculations, but was recovered only on two subsequent occasions in a total of 41 cultures. No positive cultures were obtained later than 1 week following injection.

During the course of the experiment the dog showed no definite signs of illness. No signs of peritonitis were evident. No peripheral lymph node enlargement could be detected during the experiment.

Dog was killed by intracardiac ether (5 cc.). Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog IV. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog IV, a male mongrel, weighed 8.5 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 28; agglutination for brucella 456, negative. The animal died 261 days after the first injection.

Dog IV received 28 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 19 injections were at weekly intervals; thereafter the inoculations were given irregularly, at an average of bimonthly intervals. The last injection was 7 days before death.

Temperature taken every 3 days for the first 2 months ranged between 38° and 39.5° C. Opsonocytophagic index, initially 28, varied between 44.5 and 90 during the experiment, and did not appear to be correlated with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of 1 week and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was isolated from the blood stream 1 week after the first injection, and a total of 16 times in 19 weekly cultures made during the period of weekly injec-

tions. Of 15 cultures made thereafter, 7 were positive. All positive results were obtained from cultures taken 1 week after injections; blood cultures taken at intervals of 2 or more weeks after inoculations were always negative.

During the first 12 weeks of the experiment the dog appeared well. He then developed anorexia, lassitude, weakness, and lost weight; the weekly injections were discontinued after 18 weeks because it appeared that the animal would not survive much longer. The animal improved, and injections were resumed at bimonthly intervals. It appeared that these were being withstood very well, and weekly injections were resumed, but after the fifth of these the dog showed a rapid decline in weight, appetite, and strength, and died presenting a picture of cachexia. No peripheral lymph node enlargement was noted during the experiment.

Autopsy was performed 3 hours after death. Findings are summarized in Table II.

Dog V. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog V, a male mongrel, weighed 9.0 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 20; agglutination for brucella 456, negative. The animal died 38 days after the first injection.

Dog V received 4 intravenous inoculations of brucella (Brody strain), each injection consisting of 10 billion organisms, at about weekly intervals. The last injection was 8 days before death.

Temperature taken every 3 days ranged between 38° and 40.3° C. Opsonocytophagic index, initially 20, varied between 21.5 and 51.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and ranged between 1:2560 and 1:10240. Brucella was recovered from the blood after the first inoculation, and a total of 3 times in 5 subsequent cultures; all positive cultures were obtained at intervals of 1 week after inoculations.

The animal appeared well until the 38th day when there was sudden onset of a shock-like condition and rapid death. Autopsy performed 1 hour after death revealed the stomach to be filled with impacted shavings. Other findings are summarized in Table II.

Dog VI. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog VI, a male mongrel, weighed 8.4 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative. The animal was killed 454 days after the first injection.

Dog VI received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The injections were given at almost weekly intervals over a period of 47 weeks. The last inoculation was 136 days before death.

Temperature taken every 3 days for 2 months ranged between 37.8° and 39° C. The opsonocytophagic index, initially 7, fluctuated widely during the first 6 weeks, then ranged between 53 and 86.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was never recovered from the blood stream in 43 attempts: 38 of the cultures were made at intervals of 1 week following injection; 3 were made at longer intervals; and 2 were taken 1 day following inoculation.

During the entire experiment the dog remained well. At no time was there evidence of peritonitis, and no enlargement of the peripheral nodes occurred.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog VII. Summary of Protocol

When the experiment was begun, on September 30, 1940, dog VII, a male mongrel, weighed 8.6 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 398 days after the first injection.

Dog VII received 33 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These injections were given at almost weekly intervals over a period of 38 weeks. The last injection was 132 days before death.

The opsonocytophagic index rose rapidly from 7 to a level ranging from 41 to 84 during the experiment. Agglutination of brucella 456, initially negative, rose to 1:5120 and varied between 1:1260 and 1:20480 during the experiment. Brucella was isolated from the blood stream 8 times in 34 cultures. The first positive culture was obtained after the eighth inoculation. All positive cultures were obtained after intervals of 1 week following injection.

Throughout the experiment the dog remained well. At no time was there evidence of peritonitis, nor any enlargement of the peripheral lymph nodes.

Dog was killed with intracardiac ether (1 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog IX. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog IX, a male mongrel, weighed 9 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 60; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 186 days after the first injection.

Dog IX received 3 intraperitoneal inoculations of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The inoculations were given at intervals of 10 days. The last injection was given 152 days before death.

Opsonocytophagic index varied from 58.5 to 75. Agglutination of brucella 456, initially negative, rose to 1:2560 after 1 week and varied between this dilution and 1:10240. Brucella was recovered from the blood stream once in 5 cultures. The cultures were made from 2 to 11 weeks following inoculation. The positive culture occurred 3 weeks following the second injection.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog X. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog X, a female chow, weighed 8 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 13.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 216 days after the first injection.

Dog X received 3 intravenous injections of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The injections were given at intervals of 10 days. The last injection was given 182 days before death.

Opsonocytophagic index rose from 13.5 to 42 at the end of 1 week and varied between that figure and 71. Agglutination of brucella 456, initially negative, rose to 1:10240 after 1 week, and then remained at 1:20480. Brucella was recovered from the blood stream 3 times in 5 cultures made from 2 to 11 weeks following inoculation. The first positive culture was obtained 2 weeks following the first inoculation; other positive cultures were obtained 3 and 7 weeks following the last inoculation.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac air; autopsy was performed immediately afterwards. Findings are summarized in Table II.

Experimental Results

A. Clinical Observations

From the protocols of the respective animals it is clear that a serious infection by brucella occurred in all four of the dogs inoculated intravenously with the Brody strain of organism. The infection was fatal in two of the four and doubtless would have been in a third had the intravenous inoculation been continued following the development of the profound intoxication that occurred in all of the animals in this group. The fourth intravenously inoculated animal in this group died early in the experiment from an acute intoxication produced by the ingestion of a mass of pine shavings that were being used for bedding. All of the animals of the group suffered from anorexia, pronounced loss of weight, great weakness and lassitude, and, finally, in the fatal cases, coma and death. Although the infection established in these dogs provoked the extraordinary clinical signs mentioned, it is evident from the experience with dogs II and IV that the dog has a remarkable resistance to infection by brucella and is capable of a quick and successful recovery from the acute manifestations of the disease when the inoculations are discontinued.

In spite of this great resistance to the infection, however, the organisms tend to persist in the tissues of the animal, particularly in the reticulo-endothelial system, where, as will be described shortly, they continue to provoke a morphological reaction. This is illustrated strikingly in dog II. Although this animal received the extraordinary number of 35 intravenous inoculations, the animal lived for 487 days and at autopsy was found to harbor brucella in the kidney and the lymph nodes. (No organisms had been received by this animal during the last 225 days of its life.) That the dog which has received a series of inoculations intravenously and has developed definite clinical disease is not protected against further inoculations after a rest period of considerable time is shown clearly in the experience with animal IV. This dog died following the resumption of inoculations with signs and symptoms similar to those which it had previously experienced. The disease of which these animals suffered and from which some of them died was a profound bacterial intoxication, as will be confirmed by the pathological anatomical findings to be recorded presently. This intoxication was not always accompanied by bacteremia. In fact, the

bacterial observations on these dogs showed that the dog is able to clear the blood of the inoculated organism within a relatively short time, usually within 3 weeks following the inoculation. After this time the organisms were found only in the tissues.

Judging from our experience with intraperitoneal inoculation of the dog, this route of infection is ineffective in establishing the clinical disease regardless of the number of inoculations administered. Nevertheless, blood cultures following intraperitoneal inoculations were positive on a number of occasions in two of the dogs so inoculated. In these animals the agglutination titers and the opsonocytophagic indices rose significantly, indicating a definite response of the tissues to the organism even though there were no clinical manifestations of disease. Our experience with intraperitoneal inoculation was the same for both strains of organisms employed.

It is worthy of comment that, whereas all the dogs inoculated intravenously with the Brody strain of brucella (an organism recovered from a case of Hodgkin's disease and of relatively low virulence for guinea-pigs) developed profound clinical disease, the one dog inoculated intravenously with the ABF 36 strain of brucella (an organism recovered from a spontaneous infection in a hog and of high virulence for guinea-pigs) developed no clinical evidence of infection. In spite of the absence of clinical disease in this animal, however, the organism was recovered from the lymph nodes at autopsy 182 days after the last inoculation was given. Active, subclinical infection in this animal is definitely indicated by the sustained elevation of the agglutination titer and the opsonocytophagic index, as well as by the demonstration of a positive blood culture 81 days following the last inoculation. The somewhat unexpected reaction of this dog possibly may be explained on the basis of the relatively few inoculations that were given; this animal received only 3 intravenous inoculations. This would seem to indicate that a relatively large number of inoculations, even though made intravenously, is necessary to establish clinical disease in the dog.

All of these clinical observations confirm the impression of previous workers that the dog is highly resistant to infection by brucella. At the same time, however, they indicate clearly that brucella in sufficient quantity is capable of producing even fatal disease in this animal, an observation which does not appear to have been made by previous workers.

B. Anatomical Observations

In the accompanying Table II are summarized all of the gross and histological findings in this group of animals. Of these findings only a few appear to be the result of the action of brucella. Outstanding

TABLE II

Experimental Brucellosis in Dogs: Autopsy Findings

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
I	Endocardial hemorrhage, dilatation	o	o	o	R.E.* hyperplasia pronounced; hemorrhage, iron pigment; plasma cell reaction marked; focal epithelioid reaction	Epithelioid focal granulomata; chronic diffuse glomerulonephritis	o	Severe	198 died	Brody I.V. 21
II	Focal hemorrhage, dilatation	Foreign body; focal bronchial granulomata	Epithelioid granulomata; portal hepatitis	Focal hemorrhage; R. E. hyperplasia; many giant cells of bone marrow type; reticular scarring	Hemorrhage; R.E. hyperplasia	Focal scarring; dilatation of pelvis	o	Severe for 37 wks., clinical recovery after inoculations discontinued	487 killed	Brody I.V. 35
III	Focal hyaline degeneration	o	o	o	R. E. hyperplasia	o	o	o	461 killed	Brody I. P. 39
IV	o	Bronchial pneumonia; parasitic ova	o	o	Hemorrhage; R. E. hyperplasia, moderate	Acute diffuse hemorrhagic glomerulonephritis	Severe gastritis and enteritis	Severe	261 died	Brody I. V. 28

TABLE II (*Continued*)

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
V	o	Confluent lobular pneumonia	o	Epithelioid focal granulomata	R. E. hyperplasia; granulomatous reaction	o	o	None; died from eating bedding	38 died	Brody I. V. 4
VI	Focal hyaline degeneration	o	o	Chronic capsular inflammation with scarring	R. E. hyperplasia, moderate	Focal granulomata	o	o	454 killed	Brody I. P. 39
VII	o	o	o	Capsular scarring; reticular scarring	R. E. hyperplasia pronounced	o	o	o	398 killed	Brody I. P. 33
IX	o	o	o	Reticular scarring; pulp sparse; hyperplasia of malpighian cells	R. E. hyperplasia pronounced; masses of mononuclear cells packing sinuses and replacing lymphatic cords	o	o	o	186 killed	ABF 36 I. P .3
X	o	o	o	o	R. E. hyperplasia with focal epithelioid reaction; iron pigmentation	Focal granulomata	o	o	216 killed	ABF 36 I. V. 3

* R.E. = Reticulo-endothelial.

among the various lesions are the following: (a) dilatation of the heart, (b) focal granulomata in the lymph nodes, liver, and kidney, (c) chronic inflammation and fibrous thickening of the splenic capsule, (d) hemorrhages or hemorrhagic pigmentation of the lymph nodes, (e) acute diffuse glomerulonephritis, (f) pronounced reticulo-endothelial hyperplasia in the lymph nodes, (g) intracellular brucella in the cells forming the sinusoidal reaction of the lymph nodes. Certain of these lesions are discussed briefly.

The most important of these lesions, as they relate to the primary objective of our experiment, is the rather remarkable sinusoidal reaction within the lymph nodes (Figs. 1 to 5). This lesion was found quite uniformly throughout the group of animals even though there was no gross enlargement of the lymphoid tissues. This sinusoidal reaction consisted of the proliferation of a mass of large mononuclear cells accompanied by an accumulation of a great number of polymorphonuclear leukocytes (Fig. 2). The lesion can be described best as a profound reticulo-endothelial reaction in which, as a rule, only the sinusoidal cells participate. However, in some of the nodes so affected a comparable reaction on the part of the reticulum of the lymph nodes occurred. The latter reaction resulted in the development of little foci of large, pale cells of distinctly epithelioid type (Figs. 3 and 4). Although these lesions were the exceptional finding, their presence seemed to leave no doubt that brucella is capable of exciting the growth of both the reticular and the sinusoidal elements of the reticulo-endothelial system. This reticulo-endothelial reaction, although prominent in virtually all of the nodes studied from all of the animals inoculated, is not quantitatively comparable to the reaction of the reticulo-endothelium that takes place in the lymph nodes of the guinea-pig when that animal is inoculated with brucella (Fig. 6); nor is the reaction comparable to that in the hog which we have described in a previous communication.[2] It is important to note that in the cytoplasm of these proliferating sinusoidal cells and the accompanying polymorphonuclear leukocytes, brucella in considerable quantity was demonstrated. This observation was made on the sections from an animal which had been inoculated 8 days before death (Fig. 2). In some of the lymph nodes showing the reticulo-endothelial reaction an extraordinary accumulation of plasma cells was found. These appeared in foci, usually situated outside of the sinuses and in the lymph cords. In lymph nodes showing this reaction the lesion as a whole resembled a genuine granulomatous reaction in its earliest phase of development (Fig. 5). Accompanying the reaction, multinucleated giant cells occasionally were found. These usually were not of the Langhans type. In some instances they resembled

somewhat the multinucleated giant cells seen in Hodgkin's disease. In all of the lymph nodes showing the sinusoidal reaction, variable quantities of fresh blood and iron pigment were found. Many of the sinusoidal cells contained phagocytosed materials of this sort. Accompanying these macrophages, there was always a great accumulation of the large reticulo-endothelial cells showing no evidence of phagocytosis. Although the changes described were pronounced in the lymph nodes in general, in none of these structures was there complete disturbance in the normal architectural relationships.

The little granulomatous foci in the kidney (Fig. 9), the liver (Fig. 10), and the spleen (Figs. 7 and 8) were found in only one or two of the animals. This lesion was especially prominent in the kidneys of one of the animals. Microscopically, the granulomatous focus was a collection of large mononuclear, sometimes epithelioid, cells accompanied by a few polymorphonuclear leukocytes and a few lymphoid cells. In some instances necrosis had occurred in the center of the lesion. No organisms could be demonstrated in any of these lesions. In general, the histological appearance of these little granulomata was identical with that of the foci of reticulo-endothelial hyperplasia found in the lymph cords of the lymph nodes. In view of the similarity of these lesions to those that one finds in brucellosis of the guinea-pig, and in view of the recovery of brucella by culture from nodes showing these lesions, it seems permissible to attribute the renal lesions to the brucella infection even though organisms could not be demonstrated in histological sections.

At this point it is necessary to call attention to the occurrence of focal granulomata of another type in the lungs of one of the dogs. These were quite clearly unrelated to the brucella infection; they were situated about the bronchi and always were associated with foreign bodies.

In two of the dogs the reticular structure of the spleen was particularly dense. This was unassociated with an increase in the number of pulp cells. The interpretation of this condition as a form of reticular scarring seems justifiable, but its relation to the brucella infection is difficult to establish.

The fibrous thickening of the splenic capsule occurred only in the animals inoculated intraperitoneally. Within the capsule at its thickest portion was found a mild chronic inflammatory reaction suggesting that the lesion was originally an inflammatory one (Fig. 8). Thus, it would appear that this lesion represents a brucella effect. No other alterations of the peritoneum that could be related to the inoculations were found.

All of the lesions other than those just discussed we have regarded as incidental findings or as the effects of the general intoxication produced by the long-continued brucella infection. One of these lesions, the diffuse glomerular injury to the kidney, is worthy of brief comment. This occurred in two of the animals and was unrelated to the development of the granulomatous foci. The histological changes found are like those customarily associated with acute and chronic diffuse glomerulonephritis in man. The route of inoculation in both of the animals showing this lesion was the same, that is, intravenous. These lesions are of particular interest in view of the fact that the experimental production of a typical acute diffuse glomerulonephritis by whatever method employed is a difficult accomplishment. The conditions of our experiment were such that one would have expected the development of a focal type of glomerulonephritis, a common form of injury to the kidney in all forms of bacteremia. It should be emphasized that the renal injury found in these two animals is unlike the usual spontaneous nephritis in the dog. All things being considered, it seems justifiable to attribute the nephritis in these animals to the experimental procedures. A discussion of the mechanisms involved in the production of injury to the kidney of the sort seen in these animals is not pertinent to our primary objective in this paper, and so we have dealt with the problem elsewhere.[5]

Comment

In these experiments it seems clear that we have succeeded in producing in the dog an infection by brucella that is recognizable both clinically and anatomically and which, in some instances, has been of sufficient severity to result in the death of the animal. That this has been accomplished only through the utilization of repeated inoculations of the organisms and that the anatomical alterations accompanying the infection are nondestructive and of an extremely mild character seem to indicate clearly that the dog is a highly resistant animal with tissues that are slow to react to infection by brucella. This observation is in harmony with the conclusions of previous workers. In planning our experiment, it had been hoped that the utilization of an animal that is so refractive to infection by brucella might make possible the production of long-standing chronic anatomical alterations in the tissues of the reticulo-endothelial system resembling the changes occurring in Hodgkin's disease. It is obvious that this has not been accomplished. At the same time, it is clear that a chronic infection by brucella does give rise to a pronounced alteration in the character of the lymphoid tissues of the dog. This alteration is the expression of a basic reaction on the part of the reticulo-endothelial cells that *in principle* may

be considered comparable to what occurs in Hodgkin's disease. If brucella is related etiologically to Hodgkin's disease, a possibility which certain recent observations seem to suggest, there must be certain peculiar and highly important factors involved in the relationship that the experimental studies of ourselves and others have not yet disclosed. Theoretically, it appears entirely possible that such factors may exist.

Summary and Conclusions

1. By means of repeated intravenous inoculations of a strain of *Brucella suis* obtained from a case of Hodgkin's disease, a severe, sometimes fatal, form of chronic brucellosis has been produced in dogs. Dogs so affected have been observed for as long as 487 days.

2. It has not been possible to produce clinical disease in dogs by repeated intraperitoneal inoculations of a strain of *Br. suis* obtained from a case of Hodgkin's disease. This was true also when the inoculations were made with a strain of *Br. suis* obtained from a naturally infected hog. Both of these strains of brucella were known to be pathogenic for guinea-pigs.

3. Clinical brucellosis in the dog is characterized by anorexia, loss of weight, weakness, lassitude, and coma. The course of the disease is progressive only so long as the inoculations are continued. The dog is highly resistant to the infection and may recover from the most severe infection if the inoculations are discontinued.

4. Dogs repeatedly inoculated either intravenously or intraperitoneally and without clinical disease may harbor virulent brucella in the tissues of the reticulo-endothelial system for as long as 7 months after the inoculations are discontinued.

5. The most constant anatomical alterations resulting from brucella infection in the dog are found in the lymph nodes. These consist of a pronounced reticulo-endothelial reaction involving both the sinus endothelium and the reticulum cells of the lymphatic cords, without significant enlargement of the nodes. The result of this reaction is the development of focal granulomata of epithelioid character in the lymphatic cords and the formation of great masses of large mononuclear wandering cells which fill and eventually replace the lymphatic channels. Similar focal granulomata of epithelioid character occur occasionally in the kidney, liver, and spleen. A variety of nonspecific lesions occur, including petechial hemorrhages, focal hyaline degeneration of the heart muscle, and acute gastro-enteritis. These appear to be the result of bacterial intoxication.

6. Repeated intravenous inoculations of *Br. suis* produced anatomically typical, acute, diffuse glomerulonephritis in two of four dogs.

7. Prolonged brucella infection in dogs gives rise to a marked pro-

liferative reaction on the part of the reticulo-endothelial system of a granulomatous character, but it does not produce an anatomical alteration of these tissues comparable to that which characterizes human Hodgkin's disease.

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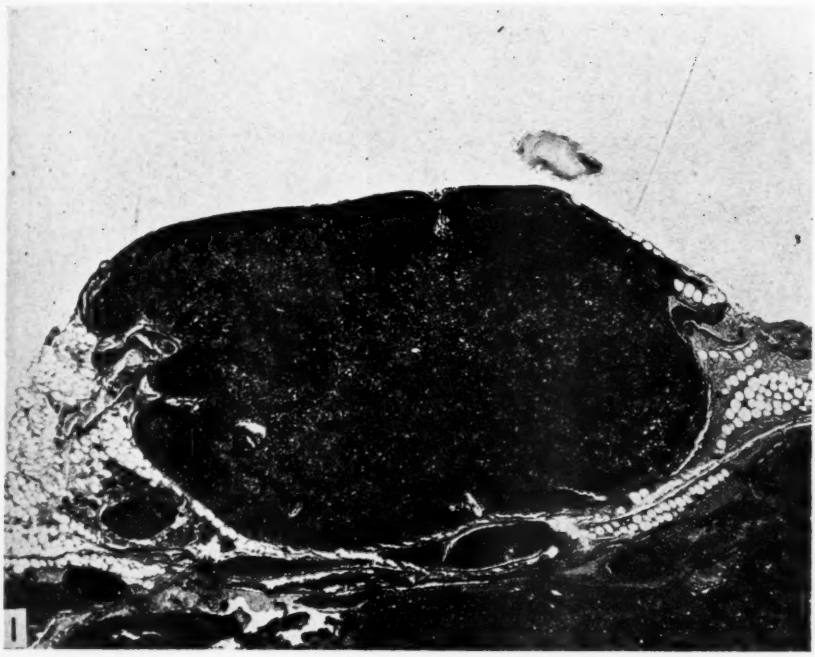
DESCRIPTION OF PLATES

PLATE 127

FIG. 1. Lymph node from dog V. This animal had received 4 intravenous inoculations of *Brucella suis* (Brody strain) which had been obtained originally from a case of Hodgkin's disease of 5 years' duration. This low-power photomicrograph shows a widespread sinusoidal reaction and replacement of the lymphoid cells by new cells. This reaction is typical of all the inoculated animals. Figures 2, 3, and 4 show the cell types involved in the reaction. × 5.

FIG. 2. Early sinusoidal reaction consisting of the accumulation of polymorphonuclear leukocytes and reticulo-endothelial hyperplasia. The lymph node is the same as shown in Figure 1. Only a few of the proliferating reticulo-endothelial cells are active phagocytes. Brucella was demonstrated in the macrophages and the polymorphonuclear leukocytes. × 485.


1

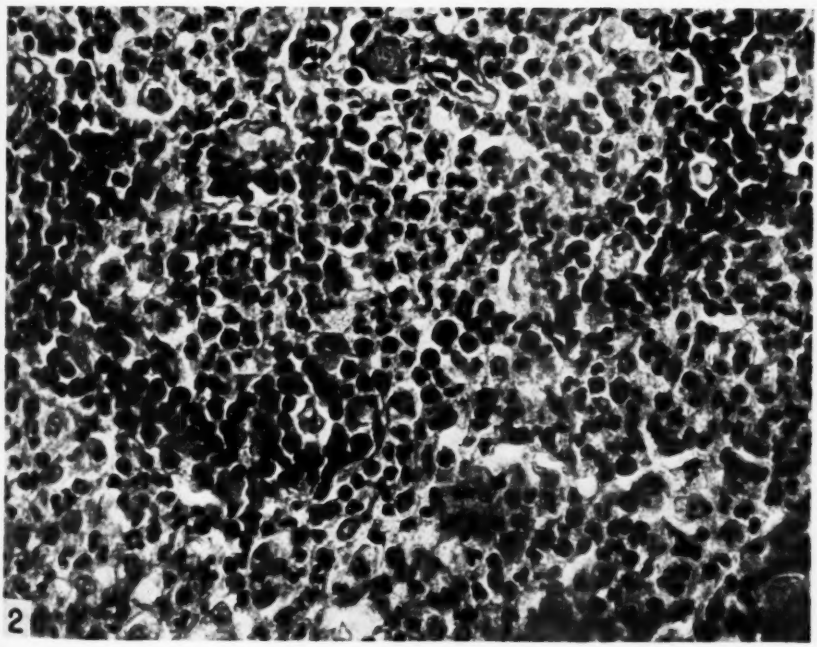

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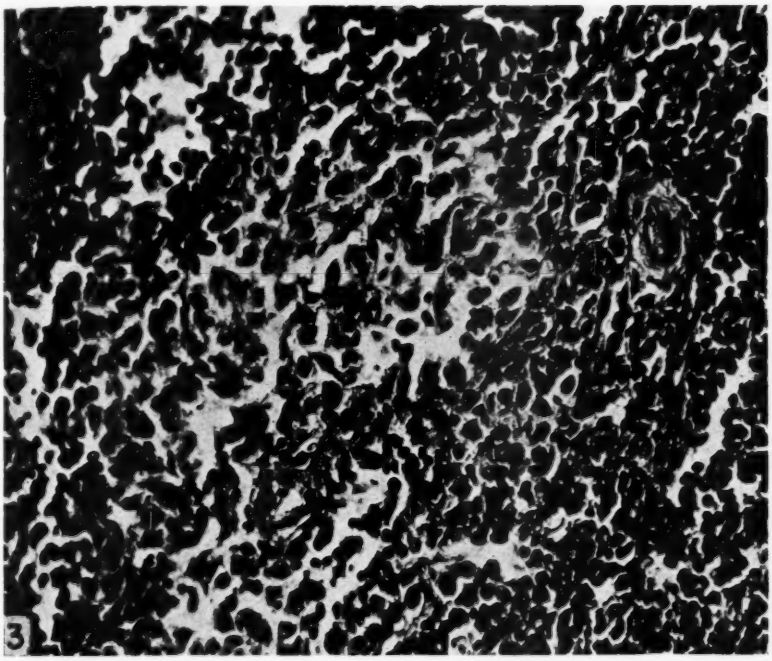
PLATE 128

FIG. 3. Epithelioid transformation of the proliferating reticulo-endothelial cells in a lymph node from dog IX. This animal had received 3 intraperitoneal inoculations of *Br. suis* (ABF 36 strain), originally recovered from a naturally infected hog. The reaction is a genuine granuloma and is identical with that which occurs in the guinea-pig (Fig. 6.) A lower power view of the node from which this photograph was made is shown in Figure 5. × 365.

FIG. 4. Focal proliferation of the reticulum cells of a lymphatic cord accompanied by a marked sinusoidal reaction in a lymph node from dog I. For comparison with the guinea-pig reaction shown in Figure 6.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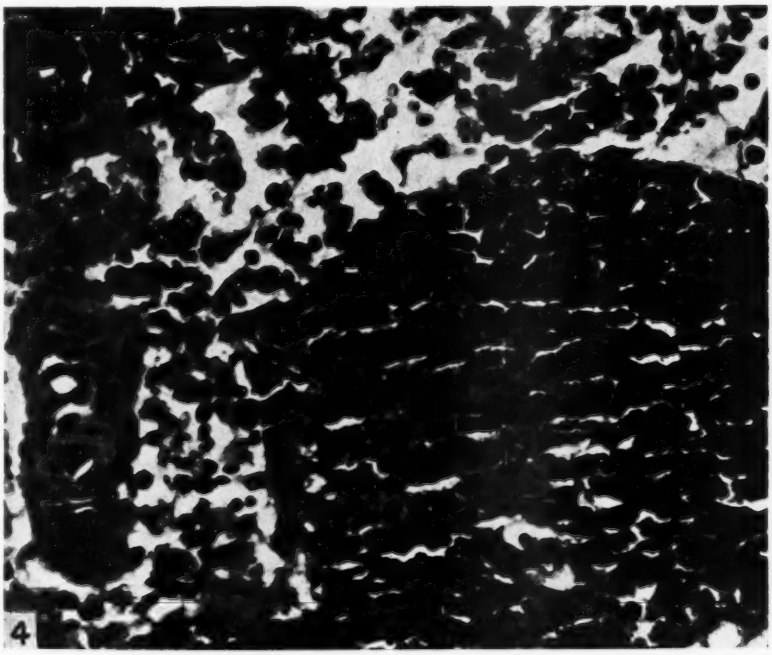
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PLATE 129

FIG. 5. Typical early reaction of the lymph nodes accompanying infection by *Br. suis* (ABF strain). This lymph node from dog IX was virtually replaced by the reticulo-endothelial reaction; grossly it resembled the node shown in Figure 1. Although the cells vary greatly in morphology, the reaction at this stage is not epithelioid except in scattered foci. These foci are pictured in Figure 3. In some areas the foci coalesce and produce a picture not unlike that in the guinea-pig reaction (Fig. 6). × 157.

FIG. 6. A typical granulomatous transformation of the lymph node in a guinea-pig infected with *Br. suis* for comparison with the reaction in the dog's node. × 137.


5

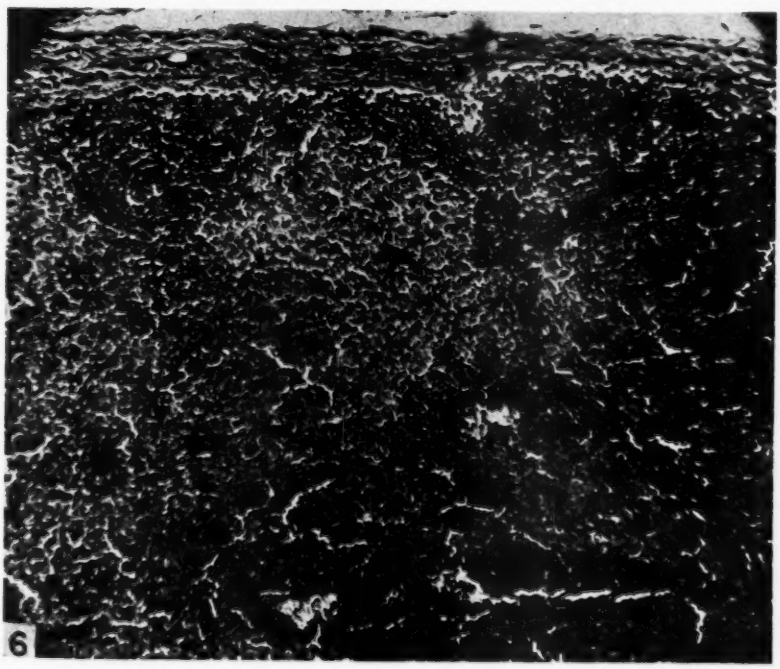
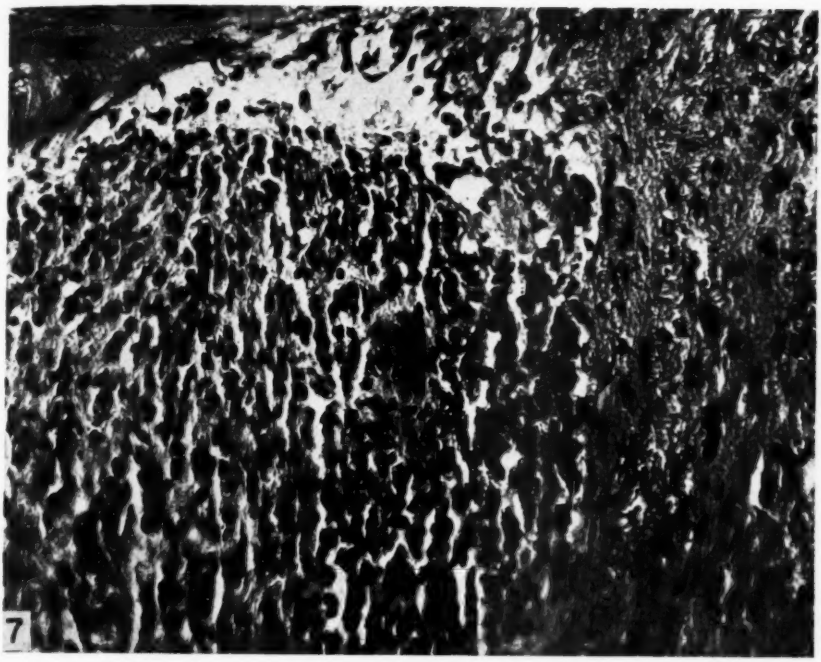

6

PLATE 130

FIG. 7. A small granulomatous focus in the spleen just beneath the capsule, with a large giant cell of megakaryocytic type. Giant cells of other types, some with one nucleus and others with several nuclei such as those pictured in Figures 2 and 4, are more common in the typical reaction to brucella. Dog VI, from which this section was taken, received 39 intraperitoneal inoculations of *Br. suis* (Brody strain) and lived for 454 days. The lymph nodes showed the reaction pictured in Figure 1, and the kidneys showed focal granulomata like that pictured in Figure 9. × 375.

FIG. 8. A necrotic inflammatory lesion in the capsule of the spleen of dog VI. The section is from the spleen shown in Figure 7. × 182.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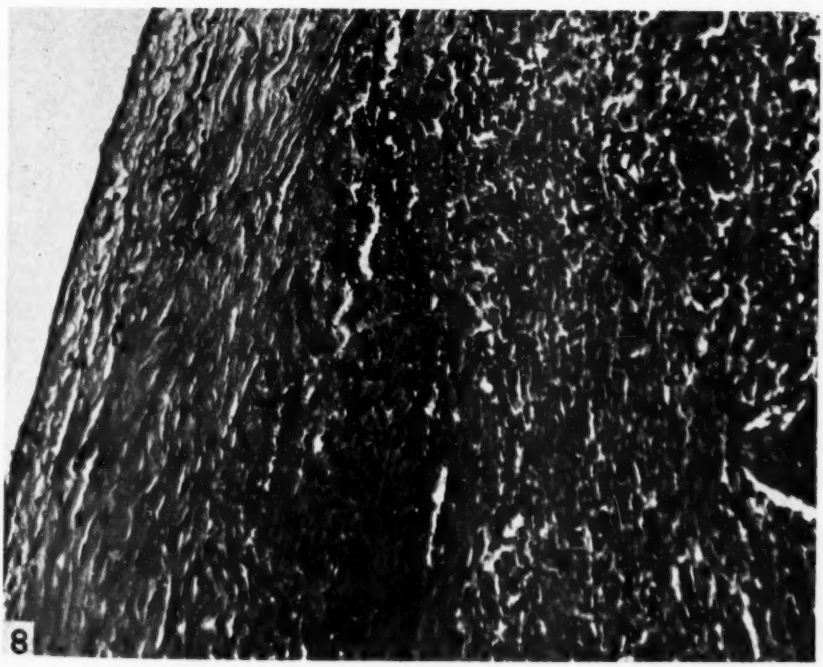
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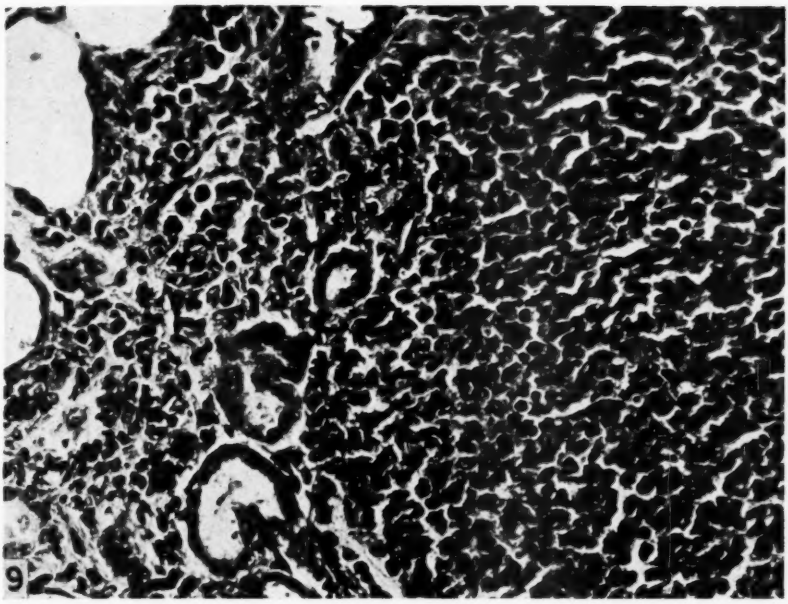
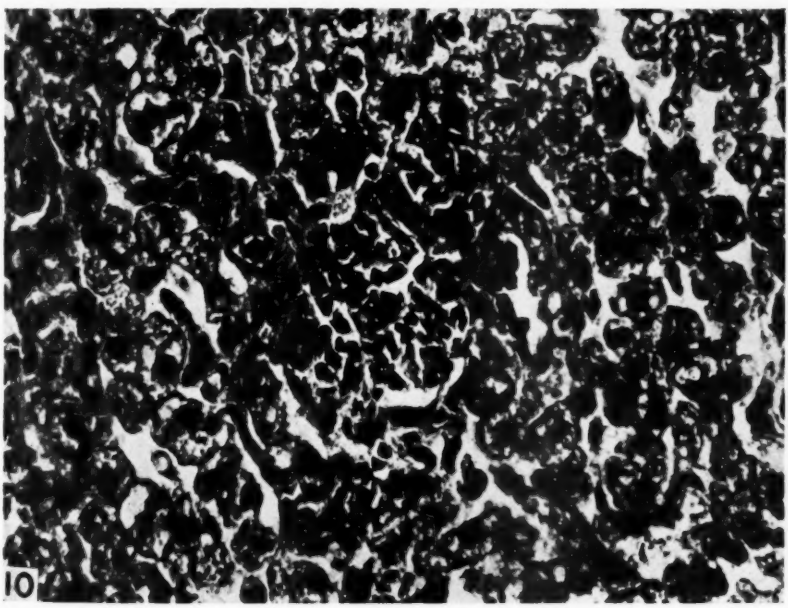
PLATE 131

FIG. 9. Focal granuloma in the kidney of dog I. The typical reacting cells are reticuloendothelial, but there is also a scattering of polymorphonuclear leukocytes; many of these are eosinophils. Lesions of this type were numerous in two of the dogs. × 365.

FIG. 10. Focal granuloma in the liver of dog II. This animal had received 35 intravenous inoculations of *Br. suis* (Brody strain) and was killed 487 days after being infected. Lesions of this sort are like those seen in the guinea-pig liver. They are not numerous. × 485.





Margolis, Forbus, and Kerby Experimental Brucellosis of Dogs

FAILURE OF PRESSOR DRUGS TO INFLUENCE "JUXTAGLOMERULAR APPARATUS" IN RATS *

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The observation of special afibrillar or granular cells in the "juxtaglomerular apparatus" of the normal kidney of various species [1-7] has led to speculation concerning the rôle of these cells. At first these cell groups were regarded as modified smooth muscle cells, somewhat analogous to myo-epithelioid cells of the myo-arterial glomus. Ruyter,[1] who first described these cells, suggested that they may modify the blood flow through the arteriole and glomerulus by imbibition and swelling, with consequent narrowing of the arteriolar lumen. Some observers [6, 7] have accepted this view. Later Elaut [8] reported hyperplasia of this cell group in dogs rendered hypertensive by denervation of the carotid sinus and cardio-aortic zones. Goormaghtigh and several coworkers found an increase in number and size of "these large, afibrillar and sometimes granulated or vacuolated cells" in renal hypertension produced by Goldblatt clamps in dogs and rabbits [9] and in experimental hypertension due to hypervitaminosis D (calciferol).[10] Goormaghtigh [11] postulated a "glandular cycle culminating in the formation of acidophil or basophil secretion granules intermingled with minute vacuoles" and concluded "that the endocrine activity of the afibrillar cells is related to the production of hypertensive substance present in the ischemic kidney." Dunihue and Candon [12] confirmed the observations in rabbits with renal hypertension [12, 13] and accepted Goormaghtigh's explanation. Hypertrophy of these cells was found by Kaufmann[14, 15] in human kidneys from hypertensive subjects. He did not find an increase in the number of cells and specified that they were large clear cells that contained a few granules, neither more definite nor distinct than they were in "normal" kidneys. Oberling,[16] in a recent report on human kidneys, objected to Elaut's and Goormaghtigh's observations on kidneys from hypertensive patients and claimed that the latter misinterpreted mastocytes and histiocytes for granular cells. Instead, Oberling reported that the preglomerular granular cells are always "degenerated or completely destroyed" in the malignant forms of hypertension.

In the absence of physiologic data supporting any of these hy-

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potheses, we undertook to test the function of the granular cells by administering pressor drugs known to act on the arterioles. Morphologic changes were sought in the character, number, and distribution of these cells as well as changes in other visceral and peripheral arterioles.

The rat was chosen because it is relatively easy to make repeated determinations of blood pressure by indirect methods and because the "juxtaglomerular apparatus" is well defined. Large granular cells are easy to demonstrate in one-fifth to three-fifths of the afferent arterioles as they enter the glomeruli in "normal" adult rats.

The blood pressure was measured at intervals before and throughout the period of drug administration to determine if chronic hypertension could be induced. As pressor substances most likely to affect the granular cells, angiotonin, tyramine hydrochloride, epinephrine hydrochloride, and pituitrin were chosen.

Angiotonin * (Page,[17] or hypertensin [18]) may be of etiologic importance in hypertension and has been shown to have special vasoconstrictor properties. Tyramine has long been suspected to be a pressor amine formed in the ischemic kidney [19-21] and its necrotizing effect on the vessels has been recorded.[22] Experiments extending from 1 to 282 days were performed with these two preparations.

Experiments were conducted for from 1 to 30 days using the more active pressor drugs. Epinephrine was used because of its constrictor effect on the renal afferent arterioles,[23-25] pituitrin because of its ability to produce vascular lesions in the kidney, stomach, and other organs.[26, 27] Another reason for examining the renal vessels was the report of augmented responses to adrenalin, tyramine, and pitressin in rabbits with renal ischemia.[28]

Methods

Altogether 50 normal rats were used, weighing from 150 to 370 gm. Males and females were about equal in number in each group. A mixed diet was fed (Purina checkers). At least three control determinations were made of the blood pressure of each animal by the indirect method of Byrom and Wilson [29] under anesthesia (sodium pentobarbital, 0.5 mg. per kg. intraperitoneally). To avoid the depressor effect of the anesthetic the animals were gently heated for 3 minutes in an environmental temperature of 40°C. during anesthesia.[30] The systolic pressure was determined twice weekly during the experimental period.

Numerous blood pressure determinations were made prior to and

* We are indebted to Dr. Irvine H. Page of the Lilly Clinic, Indianapolis City Hospital, who kindly supplied the angiotonin.

after the administration of the drug and during the pressor effect. The animals were sacrificed by inhalation of ether usually 1 hour after the last injection. Specimens of organs were fixed in Zenker's solution and serial sections through the greatest diameter of the kidney were stained with Goldner's modification * of Masson's trichrome stain and Mallory's phosphotungstic acid hematoxylin. The latter stain proved very helpful in detecting the granular cells. From different histologic sections of the kidneys of each animal twenty clearly visible afferent arterioles were studied as they entered the glomeruli. The presence of granular or clear cells was noted and any deviation in thickness or structure of the media was recorded.

Seventeen animals were given *angiotonin* in doses from 0.03 to 0.2 cc. intraperitoneally once or twice a day except Sundays for periods of 1 to 189 days. From 2 to 125 injections were administered. Interruptions of 2 to 8 days were sometimes necessary when prompt shipment of the drug was impossible.

Nineteen rats were injected with *tyramine hydrochloride* in doses of 8.75 to 25.0 mg. per kg. intraperitoneally. Injections were given once or twice daily except Sundays for periods of 2 to 282 days, to a total of 5 to 413 injections.

A 1:1000 solution of *epinephrine hydrochloride* was given to 6 animals in doses of 0.25 mg. per kg. intraperitoneally once or twice a day; 1, 5, 10, 20, and 40 injections respectively were given in periods of 1 to 30 days. In this group an attempt was made also to detect changes in the granular cells caused by the final injection of the drug. Therefore one kidney was removed before the last injection was given and the other one immediately after the blood pressure had reached its peak.

Eight animals were given 3, 9, 18, and 30 injections of *pituitrin S* (Parke, Davis & Co.) subcutaneously three times daily for 1 to 10 days. Each injection contained 5 international units.

Control experiments were done with physiologic saline solution injected intraperitoneally.

Results

The doses of the pressor drugs produced restlessness, erection of the hair, pallor of the skin of the ears and toes, an increase of respiratory rate, and, after tyramine hydrochloride, augmented salivation and often a marked exophthalmos. A steep rise of 20 to 125 mm. of Hg in the systolic blood pressure followed every injection of *angiotonin, tyramine hydrochloride, epinephrine hydrochloride,* and *pituitrin S* and reached

* Goldner, J. A modification of the Masson trichrome technique for routine laboratory purposes. *Am. J. Path.*, 1938, **14**, 237–243.

a peak within 1 to 3 minutes. The decline of the pressure was equally rapid.* Control experiments with physiologic saline solution injected intraperitoneally were without any pressor effect. The pressor response to *angiotonin* was 20 to 70 mm. of Hg; to *tyramine hydrochloride*, 40 to 113 mm. of Hg; and after *epinephrine hydrochloride*, 50 to 125 mm. of Hg. Increases which followed *pituitrin* injections ranged from 10 to 125 mm. of Hg. In none of the animals did the systolic pressure fail to return to levels recorded prior to the administration of the drug.

In neither the short nor the prolonged experiments in the four groups was there any significant change in the granular cells of the renal afferent arterioles. These cells, if present, were limited to 2 or 3 in the media at the termination of the afferent arteriole (juxtaglomerular apparatus or Polkissen †). They were counted in 20 terminal arterioles of the kidneys of each group. The 17 animals which received *angiotonin* showed granular cells present in 2 to 13 (average 8) instances; they varied from 3 to 12 (average 6) in the 19 animals of the *tyramine* group; and from 6 to 15 (average 10) in the kidneys removed just before the last injection of *epinephrine* was given. The granular cells were found in 7 to 13 (average 8) Polkissen of 20 arterioles in the kidneys extirpated after the last injection of epinephrine.

The 8 animals treated with *pituitrin S* had the lowest range, with 3 to 7 granular cells per 20 terminal arterioles; the average was 5. There was no relation between the number of granular cells and the number of injections in any of the four groups.

In 7 animals given *tyramine* the granular cells appeared swollen and were found in the proximal part of the afferent arteriole. This finding bore no relation to the number of injections, which ranged from 5 to 373 in these instances. A similar observation was made in 1 animal of the *adrenalin* series after 10 injections.

There were other histologic findings that could not be attributed to the effect of any of the drugs. More or less marked vacuolization of the medial cells in the interlobular arteries of the kidneys was very frequent and the degree could not be related to the number of injections.

In 2 animals of the *tyramine* group there were microscopic areas of

* There is a possibility that the apparent steep decline recorded by the plethysmographic method is due to progressive constriction of the tail arteries, thus yielding apparently lower readings. Intra-arterial measurements are necessary to verify this point.

† This refers to the collection of cells in the wall and sheath of the afferent arteriole visible where it enters the glomerulus. Adjacent to it is the macula densa, a palisade arrangement of the cells in the distal convoluted tubule attached to the same nephron.

focal necrosis in the myocardium, the spleen, and liver (in the latter two probably due to *Salmonella enteritidis* infection). Another animal of this group had dry gangrene at the tips of the tail, penis, and toes after 190 injections. However, no distinctive vascular lesions were present in the histologic sections of these areas.

The subcutaneous injections of *pituitrin S* caused necrotizing myositis and marked arteritis at the site of the injection. In only 1 rat, after 30 injections of pituitrin S, were there other arterial lesions found in the viscera; in this instance there was proliferation of medial and adventitial cells in some pancreatic arterioles.

Comment

The negative results presented here neither support nor deny Goormaghtigh's hypothesis of a glandular cycle in the "juxtaglomerular apparatus" with a pressor rôle in renal hypertension. They merely show that these cells are not modified by the action of pressor drugs. They also show that tyramine hydrochloride and angiotonin, given over periods as long as 282 and 189 days respectively, do not produce a permanent elevation of the systolic pressure. It is possible that by giving the same dosage over longer periods or by using larger dosage in the same period, both renal vascular changes and hypertension might be induced. The evidence in the relevant literature is inconclusive in regard to the blood pressure. Enger and Lampas [31] injected dogs intramuscularly with tyramine in amounts as large as 3.0 gm. daily for a period up to 2½ years and found increases in the basal systolic pressure of only 10 to 20 mm. of Hg; their histologic findings were negligible in all organs examined. Duff, Hamilton, and Magner [22] injected 50 to 100 mg. of tyramine into rabbits over a period of 1 to 106 days, and obtained vascular lesions in the brain, kidney, heart, larger arteries, and arterioles. They did not report measurements of the blood pressure of these animals.

Enger [32] injected 0.2 to 50 mg. of epinephrine into dogs daily up to 2 years. He found narrowing of the retinal arteries with thickening of their walls, and hyalinization of the capillaries of the glomerular tuft. Penner and Bernheim [33] in short experiments on dogs, cats, guinea-pigs, and rabbits used much larger doses than we did; they gave 0.3 to 4.2 mg. per kg. of epinephrine intraperitoneally and produced ulcerative lesions in the digestive tract of rabbits and guinea-pigs. These authors succeeded in producing bilateral cortical necrosis of the kidney when they injected 0.3 to 0.5 mg. per kg. of epinephrine into dogs for from 6 to 30 days.[34]

Dodds, Noble, and Smith [27] produced severe necrosis of the fundic

region of the stomach of rabbits by injecting 200 to 800 units of pituitrin in a single dose or a few massive doses. Using the rat they recommended the oral route of administration as the most successful and pointed out that "very large doses" are necessary to obtain gastric ulceration by subcutaneous injections (personal communication). Byrom,[26] however, injected 5 to 40 units of pituitrin subcutaneously into rats daily for several weeks and observed blanching attributed to arterial spasms of the living kidney, and infarction of kidneys and other organs, as well as necrosis in liver and arteries. Although these doses were approximately the same as those given by us, we were unable to find similar changes except for those seen at the site of injection. Nedzel [35] injected 20 units of pituitrin per 5 kg. into dogs twice weekly up to 50 injections and obtained gastric and duodenal ulcers as the result of vascular injury in 23 of 60 dogs. With the exception of the results obtained by Byrom,[26] the changes were produced either with doses considerably larger than ours or in species other than the rat.

Summary

In short (1 day) and prolonged experiments (up to 282 days), repeated doses of angiotonin, tyramine hydrochloride, epinephrine hydrochloride, and pituitrin were given to 50 rats in an attempt to influence the granular cells of the renal afferent arterioles in the so-called "juxtaglomerular apparatus."

A steep rise of the systolic pressure followed every injection but no sustained elevation above its basic level could be induced. No significant changes in the number, localization, or appearance of the granular cells or the medial cells of the renal arterioles could be found.

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PRIMARY INTRACRANIAL CHORIONEPITHELIOMA WITH METASTASES TO THE LUNGS *

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Chorionepitheliomas, which usually arise in the uterus or ovaries and less frequently in the testes, may, exceptionally, have their origin outside the reproductive organs. Most of the relatively few authentic cases of extragenital chorionepithelioma probably originated from teratomas which underwent unilateral differentiation. Intracranial teratomas, arising most frequently in the region of the pineal body, have been reported occasionally. We find, however, only two reports [1, 2] of primary intracranial chorionepithelioma.† Both cases are subject to the criticism that other possible primary sites were not adequately investigated.

The first case, which was reported by Askanazy,[1] was that of a boy, 19 years old, who had at autopsy a hemorrhagic tumor the size of a walnut in the region of the corpora quadrigemina. The tumor secondarily involved the vermis and dentate nucleus of the cerebellum. The pineal body was not identified and no metastases were noted. Histologic examination of the tumor removed from the brain disclosed a typical chorionepithelioma with cells of both syncytial and Langhans' types. The thoracic and abdominal viscera contained no tumor, but unfortunately the testicles were not sectioned. Askanazy justified his diagnosis of chorionepithelioma of the pineal body on the facts that (1) no large masses or nodules were observed in the scrotum by the clinician or pathologist, (2) pineal metastases are rare, and (3) serial sections of the pineal tumor showed no evidence of vascular dissemination. The possibility that there might have been a small primary tumor in the testicles which gave rise to a large metastasis in the brain, as mentioned by Askanazy, is not conclusively disproved. It is a well established fact that patients with small primary chorionepitheliomas may have large metastases.

The second case of chorionepithelioma reported as primary within the cranial cavity was that of Wirth.[2] The patient was a boy, 6 years

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† After this manuscript was submitted, Davidoff published a brief report of a patient with a chorionepithelioma of the pineal body (Davidoff, L. M. The endocrinologic aspects of tumors of the pineal gland. *Surgery*, 1944, **16**, 306–314). The patient, a boy, 9 years old, with signs of macrogenitosomia praecox, had a tumor in the region of the pineal body on which a diagnosis of chorionepithelioma was made from microscopical sections of the tumor, supported by a positive Friedman test. Unfortunately, post-mortem examination was limited to the head so that the possibility of primary or metastatic tumor in other organs cannot be excluded.

old, with precocious puberty. The autopsy study revealed a hemorrhagic mass with linear gray areas involving the splenium of the corpus callosum, the corpora quadrigemina, the anterior medullary vellum, and the lingula of the cerebellum. The tumor measured 28 by 48 mm. The histologic description was that of an atypical chorionepithelioma, but no microphotographs were published. There were no metastases. The left testicle was normal, but the right testicle and the thoracic and abdominal viscera were not examined. The fact that the tumor was microscopically atypical and the limited extent of the post-mortem examination leave the diagnosis of primary chorionepithelioma of the pineal body subject to considerable doubt.

In the case reported here, the diagnosis of intracranial chorionepithelioma was made ante-mortem and thorough post-mortem examination and hormone analyses were performed to establish the primary source and nature of the tumor. This case is of especial interest because it is one of the few proved instances of primary extragenital chorionepithelioma and the first with origin in the region of the pineal body definitely established.

Report of Case

The patient, a white boy, 15 years old, was admitted to the Barnes Hospital (no. 99,552) on August 8, 1942. His chief complaints were vomiting for 2 weeks, dizziness, double vision and frontal headache for 10 days, and convulsions for 3 days. He had been well until 2 weeks before, when he became listless and began vomiting. At this time he developed a staggering gait and, following lumbar puncture, it was noted that his speech was slurred. He was in a semicomatose condition when admitted to the hospital. The significant physical findings noted at that time were slight papilledema of the left eye, weakness of the left side of the face, bilateral paralysis of the sixth nerve, and diminished activity of the deep tendon reflexes. The family history, past history, and laboratory examinations showed nothing unusual. Roentgenologic examination of the skull before and after injection of air into the ventricles disclosed a calcified tumor in the region of the pineal body and a defect in the posterior part of the third ventricle.

On August 11th, a transcallosal exploration was undertaken. Because of the small size of the ventricles the exposure was very difficult. A small amount of tissue thought to be tumor was removed. Histologic examination of this tissue revealed only normal choroid plexus. Because the patient's condition failed to improve, on August 17th a Torkildsen operation[3] was performed in which a catheter was inserted between the lateral and fourth ventricles in an attempt to circumvent the block in the third ventricle. Following this operation some of the symptoms were slightly improved for a few days, but then the patient's condition became progressively worse.

On September 8, 1942, left craniotomy was performed and a solid hemorrhagic-appearing tumor was removed from the region of the pineal body. Following the operation, the patient remained stuporous and developed a huge cerebral hernia, making it necessary to tap the ventricles every few days. He then developed typical decerebrate rigidity and was fed by nasal tube. The temperature and pulse remained elevated but the respirations were regular. On October 14th, the patient

developed edema of the left leg and signs of bilateral pleural effusion, and harsh râles were heard at the bases of the lungs. Histologic examination of the tumor removed at the last operation disclosed a typical chorionepithelioma. The urine was examined and found to contain large quantities of gonadotropic hormone. The patient died on October 18th, 6 weeks after his last operation.

AUTOPSY REPORT

Autopsy (Department of Pathology, Washington University, no. 10,085) was performed 2 hours after death. The body was well developed but somewhat emaciated and there was slight edema of the left lower extremity. The nipples were more prominent than usual but there was no increase in mammary tissue. The growth and distribution of hair and the size and form of the external genitals were normal. In the scalp over the left parietal region were the healed linear wounds of the craniotomies. Beneath the scalp in this region was a fluctuant mass. On reflecting the scalp, it was found that the fluctuant mass was a part of the left cerebral hemisphere with the overlying meninges herniated through the operative defect in the calvarium. Brownish yellow fluid escaped from the herniation. Some of the fluid was collected for the determination of the presence of hormones. A small rubber catheter extended from one lateral ventricle through the calvarium and beneath the scalp to the region of the foramen magnum where it entered the fourth ventricle. A thrombus was noted in the superior sagittal sinus, but the other dural sinuses were normal.

The weight of the brain was moderately increased, being 1670 gm. The left parietal and temporal lobes of the brain were enlarged, the cerebral convolutions were flattened, and the corresponding sulci were partially obliterated. A sagittal section through the brain disclosed a mass of reddish brown neoplastic tissue completely filling the third ventricle and compressing the hypothalamus inferiorly and splenium of the corpus callosum superiorly. The tumor measured 4.5 by 4.5 by 7.0 cm., and was sharply outlined from the surrounding brain tissue (Fig. 1). As a result of its downward and posterior extension the cerebral peduncles were compressed and nearly completely transected. The surface of the tumor was finely granular and mottled red and white. The pineal body was not identified. The lateral ventricles were moderately dilated. The tentorium and falx cerebri in the region of the incisura were infiltrated with reddish brown neoplastic tissue of the same type as was noted in the brain. The spinal cord was normal and no tumor was identified during the removal of the spinous processes or around the cord.

The cut surfaces of all lobes of the lungs showed soft, white and

firm, red nodules which protruded slightly above the surrounding parenchyma. The largest nodules measured 2 cm. in diameter. Pink, friable thrombi were found in the secondary and tertiary arterial branches in all lobes. Extending to the pleural surface of the lower lobe of the right lung there was a firm, red, hemorrhagic area measuring 3 cm. in diameter. The artery leading to this area contained a red thrombus. The lower lobe of the right lung was firm, noncrepitant, and darker red than the surrounding lung.

The retroperitoneal tissues and lymph nodes were examined carefully at autopsy but no tumor was identified. After fixation the testes and epididymides were cut into slices and carefully examined without finding any discolorations, scar formation, or evidences of tumor. All abdominal and thoracic viscera were removed, fixed in formalin, and cut into 1 to 2 cm. slices in a meat slicing machine. Blocks were taken for microscopic examination from all areas suggestive of neoplasm.

Microscopic Examination

Sections of the intracranial tumor removed at autopsy presented the typical appearance of chorionepithelioma as shown in Figures 2 and 3. The tumor was composed of cells of two distinctly different types. The predominating type was a large cell with single or multiple nuclei and coarse strands and clumps of chromatin. In certain areas masses of the large cells were surrounded by the smaller second type of cell arranged in a single thin syncytial layer. This arrangement of the large and small cells resembled a chorionic villus (Fig. 2). Many extensive areas of hemorrhage and necrosis were noted throughout the tumor. The invasiveness of the tumor was shown by its penetration of adjacent brain tissue in a few areas and of the falx cerebri and superior sagittal sinus. Although sections were examined from more than 45 serial blocks of the intracranial tumor, no other evidence of a teratoma was found, and cells of the pineal body were not identified. In several of the sections there were, however, many spherical calcified bodies identical with those usually seen in the pineal body. It was concluded that the calcified bodies were of pineal origin and that all normal pineal tissue had been destroyed by the tumor.

A section through one of the hemorrhagic-appearing nodules in the lungs showed hemorrhagic, necrotic neoplastic tissue of the same type as was seen in the brain. There was invasion of the subpleural lymphatics by tumor cells and the overlying pleura was thickened and covered by a thin deposit of fibrin. The tumor was actively invading the surrounding alveoli which were partially collapsed and contained erythrocytes and a few large macrophages (Fig. 4).

A section of breast tissue showed a definite increase in the number of lining cells with an increased thickness of the ductal epithelium. A few ducts contained plugs of desquamated epithelial cells.

There was no secondary spermatogenesis in the seminiferous tubules of the testis. Between the tubules were many interstitial cells with abundant eosinophilic cytoplasm. Although microscopic sections were prepared from 47 blocks, selected from serial areas throughout the testes not more than 2 mm. apart, neither a tumor nor a scar of a healed tumor was found. There was no squamous metaplasia of the epithelium of the colliculus seminalis or utricle of the prostate gland. The pituitary gland showed a slight increase in the number of acidophilic cells.

Pathologic Diagnoses. The diagnoses were: chorionepithelioma, primary in the region of the pineal body and involving the floor of the third ventricle, with extension to the tentorium, falx cerebri, and the superior sagittal sinus; metastatic chorionepithelioma in all lobes of the lungs; hyperplasia of the mammary ducts, slight; herniation of the left cerebral hemisphere through the operative defect in the calvarium; partially organized thrombi in the pulmonary arteries, superior sagittal sinus, inferior vena cava, and left iliac and periprostatic veins; edema of the left leg, slight; recent infarct of the lower lobe of the left lung; bronchopneumonia of the lower lobes of the lungs; hydrothorax, bilateral (200 cc. on the right and 700 cc. on the left); superficial ulceration of the skin over the sacrum; lipoidosis of the aorta, slight; fatty infiltration of the myocardium; fibrous nodules in the liver; calcified nodules in a right tracheobronchial lymph node; multiloculated cystic cavities in the spleen with calcified contents.

HORMONE ANALYSES

On the day of the patient's death, specimens of urine and of cerebrospinal fluid from the lumbar region and from the lateral ventricle were collected. Four cc. of each of these solutions was injected intravenously into a virgin female rabbit. After 24 hours, several corpora hemorrhagica were present in each ovary. This indicated the presence of abnormal amounts of gonadotropins in the urine and cerebrospinal fluid.

At autopsy, 185 cc. of urine, 56 cc. of the cerebrospinal fluid, and 2.3 gm. of the metastatic tumor from the lungs were saved for analysis. The gonadotropins and estrogens were each separated from the urine according to the technic of Levin and Tyndale,[4] from the cerebrospinal fluid by the method of Delfs,[5] and from the neoplastic tissue by the procedure of Parker and Tenney.[6]

Gonadotropin determinations were made by Dr. Willard Allen by observing the response in the ovaries of mature rabbits after the injection of measured amounts of the extracts. With the methods employed a rabbit unit was found to be equivalent to approximately 5 international units of chorionic gonadotropin. The urine contained the equivalent of 310 to 435 rabbit units per liter while the cerebrospinal fluid had between 25,590 and 38,400 rabbit units per liter. The extract of gonadotropins from the neoplastic tissue of the lung had an activity of between 3,480 and 4,350 rabbit units per kg. of tissue.

The extract of the estrogenic fraction of the equivalent of 48 cc. of urine dissolved in sesame oil and injected into one ovariectomized mature rat produced proestrus changes in the vaginal epithelium. Previous observations had shown that 1.25 gammas of estrone was necessary to produce estrus in 50 per cent of the rats. The injection of the equivalent of 14 cc. of cerebrospinal fluid produced no change in the vaginal epithelial cells.

As a more sensitive test for estrogens, injections of the extracts in sesame oil were made into immature female rats about 20 days old. Using a standard technic three injections were made within 24 hours and the rats were killed 20 hours after the last injection. The change in uterine weight was determined by comparison with litter mate controls. In a similar manner, the increase in uterine weight produced by known amounts of estrone was determined. The mean body weights of comparable groups of rats varied only a few grams. Usually 3 or 4 rats were used in each group of injected and control animals. Because of the small amount of material available for analysis from the metastatic tumor, only 1 rat was injected with the total extract. These assays showed that 1,000 gm. of tumor contained the equivalent of 370 gammas of estrone. Similarly, 1,000 cc. of urine and of cerebrospinal fluids would have an activity of 90 and 30 gammas of estrone respectively.

Discussion

Of especial interest relative to this case are the hormonal secretions, the incidence and origin of extragenital chorionepithelioma in men and women, and the metastases of intracranial tumors.

Hormones

The results of the analyses of the hormones in this case are similar to the findings published by other workers. Because the fluids for analysis were inadvertently kept overnight at room temperature before the extractions were started, the results obtained may be somewhat lower than might have been found under more favorable conditions.

The amount of gonadotropins present in the urine was similar to the lower levels found in pregnant women.

Zondek [7] cited a luteinizing action of more than 416 mouse units per liter of spinal fluid as an important diagnostic point for chorionepithelioma. Each liter of this patient's cerebrospinal fluid contained between 130,000 and 190,000 international units of chorionic gonadotropic hormone. This unusually large amount of gonadotropins obtained is partially accounted for by the fact that the cerebrospinal fluid was in direct contact with the neoplastic tissue of the brain.

Zondek [7] considered 100 mouse units of luteinizing substance per gm. of extra-uterine tissue as sufficient to establish the diagnosis of metastasizing or extragenital chorionepithelioma. In this case there was the equivalent of between 17,000 and 22,000 international units of chorionic gonadotropins per kg. of pulmonary metastatic tumor. The hormones may be decreased in necrotic neoplastic tissue [7] such as was used in these analyses.

Some workers [7] have doubted that patients with chorionepithelioma have increased amounts of estrogenic substances as a result of the tumor. Other investigators,[8, 9] however, have claimed that the estrogens are significantly increased with chorionepithelioma although the amount of estrogenic substances produced is generally much less than is found in pregnant women. Twombly and Hocker [10] found more estrogens in the urine of a man with a chorionepithelioma of the testis than in the urine of any other normal male or menopausal female patient whom they tested. Gilbert [11] also reported abnormal amounts of estrogen in 7 cases of chorionepithelioma.

The urine of an adult male contains an average equivalent of 7 gammas of estrone per liter.[12] Our results show an increase of thirteen times this amount of estrogenic substances in the urine. The cerebrospinal fluid, and especially the metastatic neoplastic tissue, also contained abnormal amounts of estrogens.

Gynecomastia has been reported in 10 per cent of the cases of chorionepithelioma of testicular origin.[13] Although the estrogens may produce hyperplasia of the mammary ducts, as was observed in our patient, in this instance the amount of estrogenic substances present was either small or was present for only a short period of time, because there was no squamous metaplasia of the epithelium of the colliculus seminalis or prostatic utricle such as occurs with increased amounts of estrogenic substances. Smith and Smith [8] showed that the chorionic cells themselves, when they become neoplastic, do not contain amounts of estrogen comparable to those found in normal placentas. Whether the estrogenic hormones came from the chorionepithelioma or from the

adrenals, which were morphologically normal, is not known. The demonstration of pregnandiol in the urine suggested to Twombly and Hocker [10] that the adrenals of their patient had been stimulated abnormally and this stimulation perhaps was the explanation of the increased estrogens.

Although changes in the pituitary gland resembling those in pregnancy have been reported in many cases of chorionepithelioma, only a slight increase in the number of acidophils was observed in our case. The significance of the increased number of interstitial cells in the testicle, which has been described in chorionepithelioma and was present to a moderate degree in this case, is not understood. The absence of secondary spermatogenesis can be explained by the patient's debilitated condition and does not necessarily represent a result of excessive estrogens. Perhaps if the primary tumor had been situated where it interfered less with the patient's vital functions, he might have survived longer and have shown more advanced changes evidencing hormonal imbalance.

Extragenital Chorionepithelioma

The literature contains 26 reports of men [2, 13] and 16 reports of women [14] in whom chorionepithelioma was thought to be of extragenital origin. In most of these cases the genital organs were inadequately examined. Prym,[15] in 1927, reported an instance of a chorionepithelioma of apparent extragenital origin in which there was a small scar in one testicle that in his opinion represented a healed primary testicular tumor. However, trauma of the testicle is a frequent cause of small scars in the testis, whereas the evidence of spontaneous healing of chorionepithelioma may be questioned. It is not clearly understood how the primary growth of such a malignant neoplasm, even though it is associated with hemorrhage and necrosis, could spontaneously regress while the metastases continued to grow.

Following this report by Prym,[15] authors have become more critical and have refused to accept multiple sectioning of the testicle as indisputable proof of the absence of primary testicular tumors unless multiple microscopic sections, not more than 2 mm. apart, are prepared from various areas throughout the testicle. The reports of chorionepithelioma in which the testicles are examined according to such rigid criteria fall into three groups: (1) those which contain no evidence of tumor; (2) those which contain teratoid vestiges; [16] and (3) those with easily demonstrable testicular tumor.

Erdmann, Brown, and Shaw [13] reported 1 case and accepted the 4 published cases of Fenster,[17] Gerber,[18] Kantrowitz,[19] and Weinberg [20] as conclusively proved instances of chorionepithelioma in men, not

primary in the testicle. In all of these cases the testicles were examined by the method of serial block sections and there was absolutely no evidence of a primary tumor or scars to suggest that the primary tumor had healed. It is entirely possible that there are additional instances of extragenital chorionepithelioma among the cases reviewed by Erdmann, Brown, and Shaw in which there were insufficient data to establish the case beyond criticism.

Of the 26 possible cases of extragenital chorionepithelioma in men reported and reviewed in the literature,[2, 13] 17 were believed to be primary in the abdomen, 7 in the thorax (of which 6 were in the mediastinum), and 2 in the region of the pineal body. Prym[21] cited the observation of Greiling that all primary testicular tumors metastasize to the retroperitoneal lymph nodes as evidence against the presence of ectopic chorionepitheliomas primary in retroperitoneal tissues. However, the retroperitoneal tissues are also the frequent site for the development of teratomas and of embryonic rests derived from the urogenital fold. Of the 26 extragenital chorionepitheliomas reported in men, it was claimed that 13 were primary in the retroperitoneal tissues and 1 each in the aortic lymph nodes, lung, and liver. Thus, about one-half of the reports of extragenital chorionepitheliomas do not mention a tumor in the lymph nodes or retroperitoneal tissues as one would expect if they were all metastatic from testicular tumors.

In 1940, Berman[14] reported a case of extragenital chorionepithelioma in a woman and reviewed 15 other cases with autopsies from the literature. In almost all instances the tumor was associated with pregnancy. The fact that in 14 of these 16 cases tumor was present in the lungs suggests that it is the usual "primary" or intermediate site for the development of extragenital chorionepithelioma in women. These observations have been used to support the concept that extragenital chorionepithelioma results from the malignant transformation of chorionic tissue which has been transported to the lungs.[14]

Although extragenital chorionepithelioma could arise from teratomas or embryonic rests in women as well as in men, it is more difficult to establish indisputably such an origin in women. The distribution of the neoplastic tissue in the reported ectopic chorionepitheliomas in women is essentially the same as the location of metastases in established cases of primary genital chorionepithelioma; that is, most frequently in the lungs, liver, and brain.[22] Unlike the extragenital chorionepitheliomas in men, in no instance was neoplastic tissue found in the retroperitoneal tissues or mediastinum, which are common sites for teratomas and the two most frequently claimed sites of primary extragenital chorionepithelioma in men.

Novak and Koff [23] described a case of a woman, 31 years old, in whom examination of curettings showed chorionepithelioma. The patient subsequently died of metastatic chorionepithelioma of the brain. There was no tumor in the uterus at autopsy and one must assume that a primary uterine tumor, after metastasizing to the brain, was either removed by the curettage or regressed spontaneously. That chorionepithelioma may at times undergo spontaneous healing in women is believed by some authors.[17, 24]

Wilson [24] reported a case (no. 3) of a woman, 28 years of age, who developed neurologic signs and symptoms and died. At autopsy there was chorionepithelioma in the brain, lungs, spleen, and kidneys but no evidence of this tumor in the uterus or ovaries. In such cases it is impossible to ascertain whether there had been a primary uterine tumor which disappeared after metastases had developed or whether it developed as an ectopic tumor from trophoblastic cells transported during a previous and perhaps unrecognized pregnancy. For these reasons we will give no further consideration to extragenital chorionepithelioma in women.

Origin of Extragenital Chorionepithelioma

It is generally believed that the chorionepitheliomas primary in the testicles develop from previously existing teratomas, but in a large number of cases it is not possible to identify the teratoma. In those cases in which the teratoma is not identified it is thought that the neoplastic chorionic tissue has overgrown and completely destroyed the teratomatous elements. The concept that is generally held regarding these tumors is that they arise from the undifferentiated totipotential cells found in teratomas. Cases have been reported of chorionepitheliomas that apparently arose from teratomas in the ovary, mediastinum, and retroperitoneal tissues. Some authors [18] think that germinal rests along the urogenital anlagen may give rise to retroperitoneal chorionepitheliomas, and Staemmler [25] has reported the occurrence of testicular rests in the retroperitoneal fat at the root of the mesenteric vessels. Since the plica urogenitale extends from the sixth thoracic to the second sacral segments in the embryo, it is conceivable that germinal rests might be present in the mediastinum in the adult.

Sweet [26] reviewed 156 cases of dermoid, teratoid, and teratomatous intracranial tumors reported in the literature prior to 1940. He found a total of 44 teratomas, of which 28, or 63.6 per cent, were situated in the region of the pineal body, 4 were in the pituitary region, and 3 in the posterior part of the posterior cranial fossa. These are the three most frequent sites of intracranial teratomas. Occasionally, teratomas are

observed in the choroid plexus of the ventricles, but the more frequent sites of origin within the cranial cavity are in the region of the pineal body and of the pituitary gland.

Although the lungs and the liver are the most frequent sites for metastases from chorionepithelioma, the brain is also frequently involved. We know of only 2 cases [1, 2] in which it was claimed that a chorionepithelioma was primary within the cranium and in which no evidence of tumor was reported elsewhere in the body. As already mentioned, in these cases the examination for possible tumors of the testis was inadequate. In instances where tumor is present in other organs, as well as the brain, the burden of proof rests upon the author to show that the tumor within the brain is not metastatic.

Extracranial Metastasis of Intracranial Tumors

Metastases from primary intracranial tumors are extraordinarily rare. Several cases with extracranial metastases from intracranial fibroblastic tumors (arachnoidal fibroblastoma) have been reported.[27, 28] Instances of extracranial metastases from gliomas are less well established than from arachnoidal fibroblastomas (meningioma) although there are two reasonably well substantiated cases.[29] The case presented here is an instance of a primary intracranial tumor that produced extracranial metastases and adds interesting information to the general question of the observed infrequency of extracranial metastases from intracranial tumors. The chorionepithelioma is a tumor notorious for its ability to metastasize, and the metastases are usually blood-borne. Our case and the cases reported by Russell and Sachs [27] and Abbott and Love [28] demonstrate clearly that tumors that are known to have the ability to metastasize show this tendency when they are primary within the cranial cavity.

Discussion of Reported Case

We believe that our patient had a teratoma in the region of the pineal body from which developed a rapidly growing chorionepithelioma as a result of the differentiation of one component of the teratoma. The chorionepithelioma then destroyed the original teratoma as well as all normal tissue. The intracranial tumor invaded the superior sagittal sinus and other blood vessels and metastasized by way of the venous blood to the lungs.

In our opinion the possibility that the tumor was of testicular origin was eliminated by the careful examination of the testicles, as previously described. If a primary tumor of the testicle were present, it was less

than 1 mm. in diameter and lacked the characteristic red color of a chorionepithelioma or the firm white consistency of a scar of a healed tumor. A most careful search of gross sections of all of the other organs, which were cut into slices of 1 to 2 mm. in thickness, showed that no tumor more than 2 mm. in diameter was present except in the brain and lungs.

That the tumor nodules in the lungs were metastatic rather than primary is indicated by the absence of demonstrable tumor in the mediastinum or near the hilum of the lungs where teratoma and extragenital chorionepithelioma have been reported. Moreover, teratomas are rare tumors of the lungs and the peripheral location of the tumor nodules is characteristic of metastatic tumor. The demonstration of neoplastic cells within the veins about the tumor in the brain offers an obvious explanation of the manner of the production of the pulmonary metastases.

In favor of the pineal body as a site of the primary tumor, in addition to the negative evidence of any other primary source, is the fact that teratomas of a type known to give rise to chorionepithelioma are frequently observed in the region of the pineal body. The observation of a single focus of metastatic tumor of the brain located in the pineal body would be most unusual. For these reasons the case here reported is thought to be an authentic example of an extragenital chorionepithelioma, an established case of primary intracranial chorionepithelioma, and one of the few examples of primary intracranial tumor with extracranial metastases.

Summary and Conclusions

The case of a 15-year-old boy with a primary chorionepithelioma in the region of the diencephalon with metastases to the lungs is reported. The histologic structure of the tumor was typical of chorionepithelioma. Quantitative analyses of hormones showed significant amounts of gonadotropins and estrogens in the urine, cerebrospinal fluid, and metastatic neoplastic tissue. It is believed that the chorionepithelioma arose in the region of the pineal body from a previously existing teratoma. The testes and other organs were eliminated as sites for a possible primary growth of the tumor by thorough sectioning.

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DESCRIPTION OF PLATE

PLATE 132

FIG. 1. Medial sagittal section of the brain, showing a large hemorrhagic and necrotic tumor of the diencephalon.

FIG. 2. Section from the surgically removed portion of the tumor in the pineal region showing structures resembling chorionic villi composed of cells of syncytial and Langhans' types. Hematoxylin and eosin stain. × 430.

FIG. 3. Section from the tumor in the pineal region, obtained at autopsy, showing syncytial cells with large hyperchromic nuclei and the numerous mitotic figures in Langhans' cells. Hematoxylin and eosin stain. × 395.

FIG. 4. The hemorrhagic, metastatic chorionepithelioma is invading the parenchyma of the lung and the subpleural lymphatics. Hematoxylin and eosin stain. × 70.

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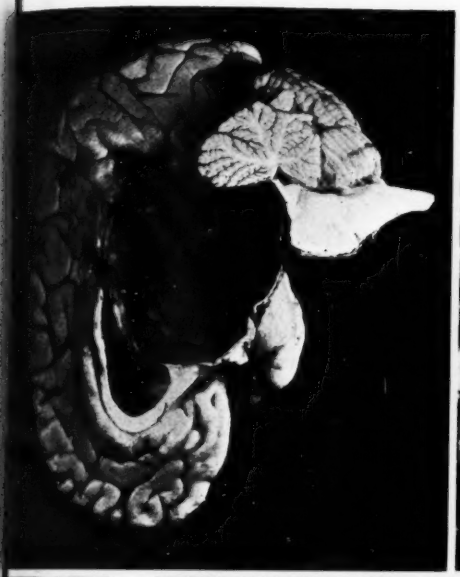

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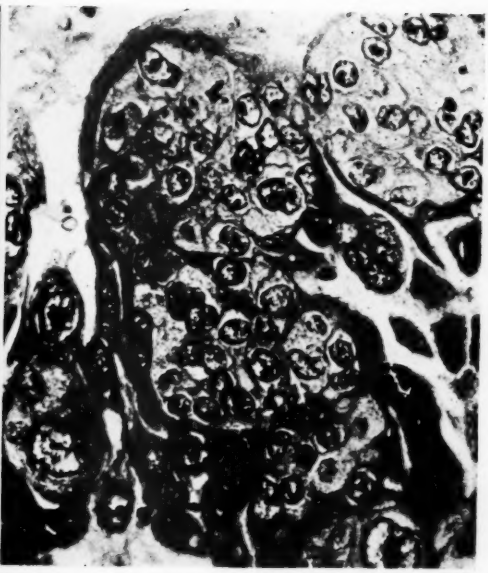

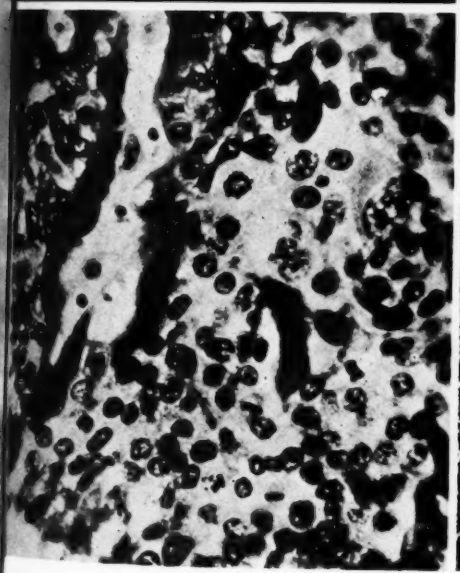

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4

Stowell, Sachs, and Russell

Primary Intracranial Chorionepithelioma

RENAL INJURY IN THE RAT FOLLOWING THE ADMINISTRATION OF SERINE BY STOMACH TUBE *

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The kidneys of animals have been injured experimentally by various means. Among the studies on this subject are a fairly large number utilizing various dietary factors as nephrotoxic agents. Weanling rats placed on diets low in choline have developed degenerative kidney disease, and Griffith and Wade [1-3] have shown that injury in this instance can be prevented by the addition of choline to the diet. Recently György and Goldblatt [4] found that the specific injurious effects of choline-deficient diets on the kidney were aggravated by the addition of pyridoxine. Hartwell [5] and also Cox and Hudson [6] have produced typical renal abnormalities in young rats by feeding them diets deficient in vitamins B. Newburgh and Curtis [7] have shown that renal lesions can be produced in animals by feeding them large amounts of certain kinds of proteins. Employing rabbits and dogs, Newburgh and Marsh [8] found that the intravenous administration of the amino acids, arginine, aspartic acid, lysine, histidine, tyrosine, tryptophane, and cystine, resulted in severe renal necrosis, while other amino acids gave no evidence of a necrotizing action on renal tissue. Later Lillie,[9] utilizing the material of Sullivan, Hess, and Sebrell,[10] found that lysine, tyrosine, tryptophane, cystine, and glutathione were nephrotoxic to young rats maintained on a diet containing 4 per cent casein as the main source of protein. The nephrotoxic action of free cystine in the diet of young rats had been previously demonstrated by Cox, Smythe, and Fishback.[11]

In the course of experiments on the dietary factors affecting the composition of phospholipids in tissues, Fishman and Artom [12] noted an injurious action of *dl*-serine administered by stomach tube in rats maintained on a synthetic diet deficient in protein and in the B vitamins. This injury was characterized by anorexia, sudden loss in weight, marked weakness and a high mortality. Similar animals on a stock diet showed only transitory and slight ill effects as a result of the administration of the amino acid.

The purpose of this paper is to report a series of anatomical studies in white rats to which the amino acid, serine, was administered by stomach tube. The report deals further with the influence of a diet deficient in B vitamins and in protein on the experimental lesions.

Recent attempts at intravenous alimentation with mixtures of amino

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acids and of protein hydrolysates add interest to the investigation of the possible toxic action of individual amino acids.[13] Further, the resemblance of some of the experimental renal lesions to certain types of degenerative kidney disease in man is of interest and may possibly have an etiological significance.[4]

Material and Methods

White male rats weighing 95 to 105 gm. were divided into four groups. The rats in group 1 (Table I) were maintained on a stock diet (Rockland Farms Rat Diet "Complete") * and received daily 100 mg. of *dl*-serine † in 3 cc. of water by stomach tube. The majority of the

Table I

Stock Diet Plus Serine

Rat	Total days on experiment	No. days receiving serine	Degenerative kidney lesions*	Liver, fatty infiltration
A–21–1	1	1	+	–
A–21–2	1	1	+	–
A–25–1	3	3	+	–
A–25–2	3	3	+	–
A–21–3	7	7	+	–
A–21–4	7	7	+	–
A–25–5	11	11	+	–
A–21–6	11	11	+	–
A–13–1	23	14	+	–
A–13–2	23	14	+	–
A–13–3	33	14	+	–
A–13–4	33	14	+	–

* See description in text.

animals were sacrificed at various intervals from 1 to 14 days after the first administration of the amino acid. Other animals which had received *dl*-serine for 14 days were allowed to continue on the stock diet alone and were killed 9 and 19 days respectively, after the last administration of the amino acid.

The second group of animals (Table II) was placed on an experimental diet (diet 4) [14] composed of "Labco" vitamin-free casein,‡ 10 parts; dextrin, 37; sucrose, 37; Crisco,§ 5; cod-liver oil, 5; "Ruffex," || 2; salt

* Rockland Farms Rat Diet "Complete" is a diet composed of ingredients of both animal and plant origin (cane molasses, soy bean meal, fish and meat scraps, various grain preparations, milk products, seed oils, yeast, etc.) The chemical composition is protein, 24.8 per cent; fat, 4.7 per cent; carbohydrate, 49.3 per cent; fiber, 4.8 per cent; ash, 9.5 per cent. Further details may be obtained from the Arcady Farms Milling Co., Chicago, Ill.

† Pure racemic *dl*-serine, $CH_2OH.CHNH_2.COOH$ was obtained from Merck and Co., Rahway, N.J.

‡ Labco vitamin-free casein is a highly purified casein which is guaranteed to be free of all vitamins and is made by the Borden Co., New York, N.Y.

§ Crisco is a partially hydrogenated vegetable oil made by Proctor and Gamble, Cincinnati, Ohio.

|| Ruffex is a cellulose material containing no fats, vitamins, or proteins and is made by the Fisher Scientific Co., Pittsburgh, Pa., especially for use with experimental diets.

mixture (Osborn and Mendel *), 4. In order to bring about as uniform experimental conditions as possible they received in addition 3 cc. of water daily by stomach tube for a maximum period of 14 days. The

TABLE II

Experimental Diet Alone

Rat	Total days on diet	Degenerative kidney lesions	Fatty infiltration of liver
A-24-1	8	Intracytoplasmic hyalinization of tubular epithelium	−
A-24-2	8	Intracytoplasmic hyalinization of tubular epithelium	−
A-24-3	13	Minimal chromatolysis of tubular epithelium	−
A-24-4	13	Minimal chromatolysis of tubular epithelium	−
A-24-5	20	Minimal chromatolysis of tubular epithelium	−
A-24-6	20	Minimal chromatolysis of tubular epithelium	−
A-23-4	20	Calcification, fibroblastic and mononuclear response	+
A-23-5	20	Calcification, fibroblastic and mononuclear response	+
A-4-1	30	Calcification	+
A-4-4	30	Calcification, diffuse cortical necrosis	+
A-5-2	40	Calcification, marked	+
A-5-3	40	Calcification, minimal	−
A-5-4	40	Normal kidneys	+

rats were sacrificed at varying intervals, after having been on the experimental diet for periods of time ranging from 8 to 40 days.

The third group of animals (Table III) was placed on the experi-

TABLE III

Experimental Diet Plus Serine

Rat	Total days on experiment*	No. days receiving serine	Degenerative kidney lesions	Fatty infiltration of liver
A-20-1	1	1	Necrosis, marked	−
A-20-2	1	1	Necrosis, marked	−
A-12-1	3	3	Necrosis, early calcification	+
A-12-2	3	3	Necrosis, early calcification	+
A-11-2	4	4	Necrosis, early calcification	−
A-11-3	4	4	Necrosis	+
A-12-6	4	4	Necrosis, calcification	+
A-12-3	6	6	Necrosis, calcification, repair	+
A-12-5	6	6	Necrosis, calcification, dilated tubules, repair	+
A-12-4	6	9	Necrosis, calcification, dilated tubules, repair	+
A-20-4	14	14	Calcification, repair nearly complete	−
A-11-1	23	14	Calcification, repair nearly complete	+
A-11-4	23	14	Calcification, repair nearly complete	−
A-11-5	33	14	Calcification, areas of scarring	+
A-11-6	33	14	Calcification, areas of scarring	+

* Not including the first 7 days on the experimental diet.

* Osborne, T. B., and Mendel, L. B. The relation of growth to the chemical constituents of the diet. *J. Biol. Chem.*, 1913, **15**, 311–326.

mental diet described above and allowed to continue for a period of 7 days. The administration of 100 mg. of *dl*-serine by stomach tube was then started and the amino acid given daily for a maximum of 14 days. The animals were then sacrificed at varying intervals, and several rats were allowed to continue on the diet for 2, 9, and 19 days after the amino acid had been discontinued.

The fourth group of animals, consisting of 4 rats, was utilized as controls; they were maintained on the stock diet until they had reached a weight of 100 gm. or more and were then sacrificed.

In agreement with previous results,[12] several of the animals of group 3 died during the first week of serine administration. No deaths occurred in the rats of the other groups. The surviving animals were killed by decapitation, and autopsy was performed immediately. Multiple blocks were taken from all organs, and fixation was done in duplicate in 10 per cent formalin and in a bichromate-formol solution. The tissues were sectioned at 6 μ and stained routinely with hematoxylin and eosin. Masson's trichrome stain and Sudan IV were employed when indicated.

Results

Anatomical changes in the kidneys

Stock Diet Plus Serine

Rats on the stock diet receiving serine by stomach tube showed a slight reduction in the expected weight gain during the second week of the experiment, but otherwise no change was noted during life. At autopsy there was no evidence of gross abnormality in any of the animals, and the organs were of their expected weight.

In those animals sacrificed 24 hours after receiving their initial supplement of serine by stomach tube, acute renal necrosis was noted at the junction of the cortex and medulla (Fig. 1). The tubular epithelium in this area was almost completely necrotic, although the general pattern of the renal architecture was maintained. The interstitial tissue, blood vessels, and glomeruli were spared for the most part. Necrosis in most instances was complete, but an occasional tubule could be seen which was not altered structurally.

The renal lesions seen in animals sacrificed after their third supplement of serine did not differ markedly from those in the rats examined on the first day. The rats killed on the seventh day, however, presented kidneys almost entirely devoid of necrotic tissue, the degenerated tubular epithelium having been almost completely removed (Fig. 2). The tubular cells were replaced by elongated and flattened cells which formed the lining epithelium of greatly dilated tubules. Removal of the

necrotic tissue was thorough in the kidneys of animals sacrificed after their eleventh supplement of serine, so that only a very occasional fragment of degenerated tubular epithelium could be found.

The kidneys of the animals which were allowed to continue on the stock diet for 9 days following the last administration of serine showed almost complete repair, small atrophic tubules surrounded by active fibrous tissue cells and an occasional dilated tubule being the only remaining evidence of antecedent renal injury. Animals sacrificed 19 days after serine was withdrawn showed further evidence of repair in the kidneys, only a very occasional dilated tubule being seen, together with small areas of scarring containing small atrophic tubules.

Experimental Diet Alone

Animals maintained on the experimental diet failed to gain weight in the normal fashion but at autopsy presented no gross pathological lesions. The microscopical findings in the kidneys were not striking. Rats sacrificed between the eighth and twentieth days of the experiment showed minimal cellular changes in the tubules. Intracytoplasmic hyalinization and fatty degeneration were seen in certain of the convoluted tubules, and careful study revealed chromatolysis progressing to complete necrosis in certain instances. These changes were not seen in every animal, and in no instance were they pronounced.

Two of the animals sacrificed after having been on the experimental diet for a period of 20 days showed, in sections stained with hematoxylin and eosin, easily demonstrable lesions in the form of fine bluish granules, which had the microscopical appearance of calcium, within the tubular epithelial cells. The tubules involved in the process were surrounded by fibrous tissue which was infiltrated with mononuclear cells. Rats sacrificed on the thirtieth day of the experiment presented definite areas of calcification within the tubular epithelium, the granules having been replaced by laminated sheets of bluish staining material. One animal presented diffuse cortical necrosis in addition to the tubular calcification (Fig. 3). Of the 3 rats allowed to continue on the experimental diet for 40 days, 2 showed areas of tubular calcification, while 1 animal presented no lesion demonstrable histologically.

Experimental Diet Plus Serine

Animals placed on the experimental diet for a period of 7 days before receiving serine by stomach tube showed marked renal necrosis within 24 hours after the initial administration of the amino acid (Fig. 4). The renal injury was similar to that which followed the administration of the amino acid to animals on the stock diet but was more

severe. Necrosis was most marked in the innermost portion of the cortex, but sometimes extended toward the periphery to involve the descending portions of the proximal convoluted tubules. Following the third supplement of serine, in sections stained with hematoxylin and eosin, bluish granules appeared within the cytoplasm of the tubular epithelium, and even at this early period the deposit was extensive (Fig. 5). The picture varied somewhat in different animals, but in general was progressive. By the sixth day after the beginning of serine administration, the granules had been replaced by definite flakes of calcium (Figs. 6 and 7). In addition, the necrotic débris had been almost completely removed, and there was considerable fibroblastic activity associated with mononuclear, eosinophilic, and neutrophilic infiltration in the immediate vicinity of the renal injury (Fig. 8). From this point on the picture was that of a rapid repair, so that the kidneys of animals sacrificed on the ninth day of serine administration showed almost complete disappearance of mononuclear and polymorphonuclear cells, and very little necrotic tissue could be seen. An occasional dilated tubule was visible, and numerous small atrophic tubules were seen in the areas of scarring. In the kidneys of animals sacrificed on the fourteenth day of serine administration, numerous small, well defined scars containing areas of calcification were seen (Fig. 9). Kidneys removed from the animals sacrificed on the thirtieth day of the experiment showed a continuation of the healing process, with areas of tubular hypertrophy alternating with areas of renal scarring (Fig. 10). The picture closely resembled that of the finely granular kidney seen in various types of renal disease in man, the areas of hypertrophy and scarring being similar to those seen in the arteriolosclerotic kidney and also in chronic glomerulonephritis. The kidneys of animals sacrificed on the fortieth day of the experiment presented a similar, but more advanced, picture (Figs. 11 and 12).

Stock Diet Alone

Animals maintained on the stock diet until they had reached the desired weight showed no demonstrable renal lesion.

ANATOMICAL CHANGES IN ORGANS OTHER THAN THE KIDNEYS

Animals maintained on the experimental diet for a period of 8 days showed an increase in liver fat which varied considerably in amount. By the twentieth day of the experiment, marked fatty infiltration was seen in the liver. The livers of rats maintained on the experimental diet supplemented with serine did not differ markedly from those seen in

animals maintained on the experimental diet alone. However, the majority of the animals in group 3 which died during the first few days of the experiment showed degenerative changes in the liver cells around the central veins, associated with marked congestion in that area. In those animals which were fed the stock diet alone, or the stock diet supplemented with serine, no abnormal changes in the liver were noted.

Myocarditis, which in most instances was confined to the auricles, was present in approximately one-third of the animals. It was seen in all four groups of rats with approximately the same incidence.

Those animals which succumbed following serine administration during the early stages of the experiment showed acute capillary dilatation which in many instances had proceeded to transudation.

Discussion

Twelve rats maintained on a stock diet and receiving serine by stomach tube showed in every instance marked renal necrosis followed by rapid removal of the necrotic tissue and almost complete repair. The necrosis was extensive and developed rapidly, being seen in animals sacrificed 24 hours after their initial supplement of serine. The process of repair was also remarkably rapid, the necrotic tissue being almost completely removed from the kidneys by the seventh day of the experiment. It is apparent further that the continued administration of serine after the initial injury does not maintain or augment the process. Kidney repair progresses rapidly and is almost complete regardless of whether serine is continued or not.

Rats maintained on an experimental diet deficient in protein and in the B vitamins developed minimal degenerative changes in the tubular epithelium which were followed by calcification. These changes were in no instance pronounced, although they were definite and occurred in the same anatomical position in the kidney as did those lesions which followed serine administration.

Animals maintained on the experimental diet supplemented with serine by stomach tube showed renal necrosis similar to that seen in the rats fed a stock diet supplemented with serine, but more extensive. In addition, there were present on the second day following serine administration bluish intracytoplasmic granules in sections of the kidneys stained with hematoxylin and eosin. Later these granules became incorporated into rather extensive calcium deposits. Here again, the continued administration of serine did not appear to augment the initial lesions induced by the first supplement of the amino acid. The renal damage appeared to be acute and progressed rapidly to its maximum

proportions. Repair was initiated early and proceeded rapidly, but in most instances the renal injury had been so extensive that the kidney could not return to normal.

It is apparent from these experiments that, coincident with renal injury, there was capillary dilatation which in some instances was followed by passage of fluid through the vessel walls. In those animals which survived the initial renal insult, the vessels appeared to regain their tone, and these rats showed no evidence of peripheral circulatory failure. The viscera of rats dying during the stage of acute renal necrosis, however, showed marked dilatation of the capillaries and hemorrhage into the tissue spaces. This was most marked in the lungs and liver but was present in all organs. Occasionally, free fluid was seen in the serous cavities. One can conclude with reasonable safety that peripheral circulatory failure was the mechanism of death in these animals.

Rats placed on an experimental diet deficient in proteins and also in B vitamins showed, in frozen sections stained with Sudan IV, a progressive increase in the amount of hepatic fat. Finally, in sections stained with hematoxylin and eosin, large vacuolated spaces could be seen within the hepatic cells. Since the experimental diet was poor in choline or choline percursors, this was expected. The addition of serine to the diet did not appear to accentuate the process, for the livers of the animals in the third group did not differ markedly in fat content from those of the animals in the second group.

A low-grade myocarditis, confined for the most part to the auricles, was present in a fairly large percentage of the animals employed in these experiments. There was no correlation between the cardiac lesions and the experimental procedure employed in the present series.

In our studies it has been shown that serine administered by stomach tube exerts an injurious effect on the kidneys of rats. Many authors who have produced renal injury with amino acids, however, have used the parenteral route. An exception is the work of Cox, Smythe, and Fishback,[11] who found that free cystine in the diet was nephrotoxic in young rats weighing 60 gm. or less, but that older rats on the same regimen failed to develop signs of renal injury. Also, other amino acids have been shown to be nephrotoxic in young rats maintained on a diet deficient in protein.[7, 8] In this connection, it may be noted that in the present study older rats (approximately 100 gm.) were employed.

Summary

Rats maintained on a stock diet supplemented with serine by stomach tube showed severe renal necrosis within 24 hours after the initial supplement of amino acid. The administration of serine beyond the

first few days of the experiment did not appear to augment the necrotizing process.

The injurious effect of serine on the kidneys of rats can be greatly augmented by placing the animals on an experimental diet deficient in protein and in the B group of vitamins. Those animals which were on the experimental diet supplemented with serine showed much more extensive necrosis, followed by calcification, than did those animals receiving serine as a supplement to a stock diet which was considered adequate.

Animals maintained on an experimental diet showed rapidly progressive fatty infiltration of the liver. As this experimental diet was poor in choline or choline precursors, such fatty infiltration of the liver was expected. This finding was not modified by the administration of *dl*-serine.

Mononuclear infiltration of the myocardium was seen in all groups of rats employed in this experiment and was not considered significant. Some rats maintained on the experimental diet, which were receiving serine by stomach tube, died during the experiment. The mechanism of death here appeared to be peripheral circulatory failure.

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DESCRIPTION OF PLATES

PLATE 133

FIG. 1. Section of the kidney of an animal maintained on the stock diet and sacrificed 24 hours after receiving the first supplement of serine. The tubular epithelium is almost completely necrotic. Hematoxylin and eosin stain. × 100.

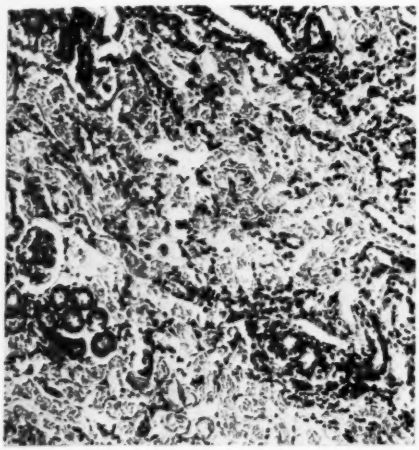
FIG. 2. Section of the kidney of an animal maintained on the stock diet and sacrificed after having received the seventh supplement of serine. The necrotic material has been almost completely removed. The tubules are dilated and are lined by flattened epithelial cells. Hematoxylin and eosin stain. × 100.

FIG. 3. Section of the kidney of a rat maintained on the experimental diet alone for 30 days. Cortical necrosis is diffuse and marked, and the calcium deposits are much more prominent in this animal than in other rats maintained on the experimental diet alone. Hematoxylin and eosin stain. × 8.

FIG. 4. Section of the kidney of a rat maintained on the experimental diet for 7 days and sacrificed 24 hours after the first supplement of serine. There is complete necrosis of the tubular epithelium in certain areas. Hematoxylin and eosin stain. × 100.

FIG. 5. Section of the kidney of a rat maintained on the experimental diet and sacrificed on the third day of serine administration. In addition to necrosis, extensive bluish granular deposits are seen within the tubular epithelium. Hematoxylin and eosin stain. × 100.

FIG. 6. Section through the entire kidney of an animal maintained on the experimental diet and sacrificed on the sixth day of serine administration. The granular deposit is extensive. Hematoxylin and eosin stain. × 8.



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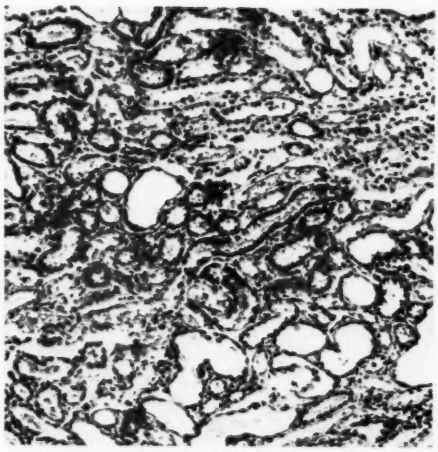

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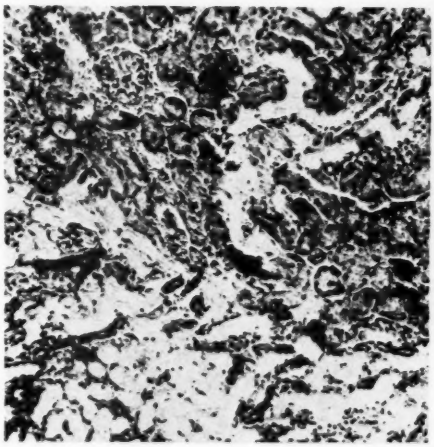

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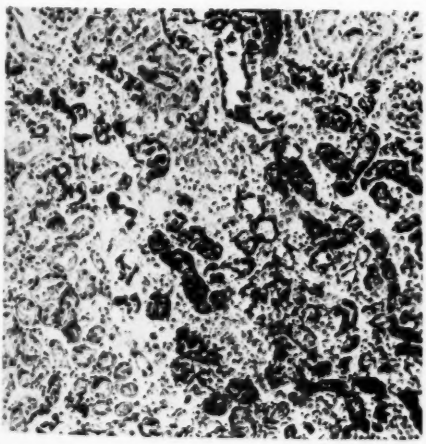

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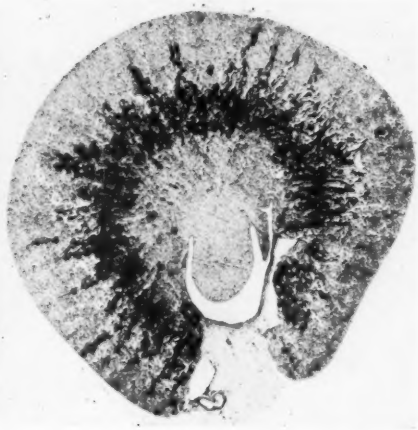


6

Morehead, Fishman, and Artom

Renal Injury Following Administration of Serine

PLATE 134

FIG. 7. A higher magnification of the kidney shown in Figure 6, showing extensive cellular infiltration. Hematoxylin and eosin stain. × 100.

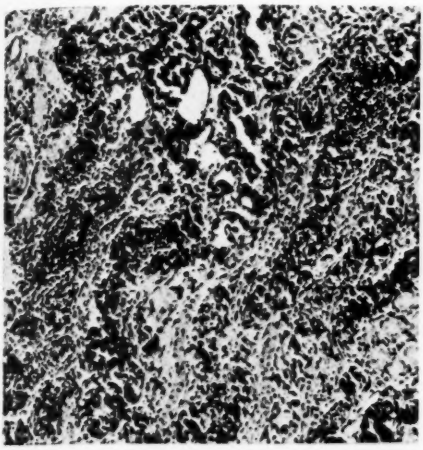
FIG. 8. Further magnification of the kidney seen in Figure 6. The infiltrating cells are both mononuclear and neutrophilic in type. Fibroblastic activity is marked, and a mitotic figure can be seen in the upper center of the photomicrograph. Hematoxylin and eosin stain. × 370.

FIG. 9. Section of the kidney of an animal maintained on the experimental diet and sacrificed after the fourteenth day of serine administration. The granules have become confluent. Large amounts of kidney parenchyma have been destroyed. Hematoxylin and eosin stain. × 100.

FIG. 10. Section of the kidney of a rat maintained on the experimental diet supplemented with serine and sacrificed on the thirtieth day of the experiment. Well defined calcium deposits may be seen. Hematoxylin and eosin stain. × 100.

FIG. 11. A section through the kidney of a rat maintained on the experimental diet supplemented with serine and sacrificed on the fortieth day of the experiment. Here may be seen the greatly dilated and hyperplastic tubules in the cortex and also the extensive calcium deposits and scarring at the junction of the medulla and inner stripe of the cortex. Hematoxylin and eosin stain. × 8.

FIG. 12. A higher magnification of the same section as used for Figure 11, showing the extensive destruction of the renal parenchyma and its replacement by scar tissue. Hematoxylin and eosin stain. × 100.



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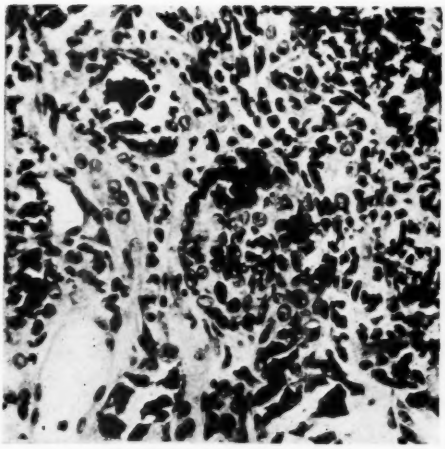

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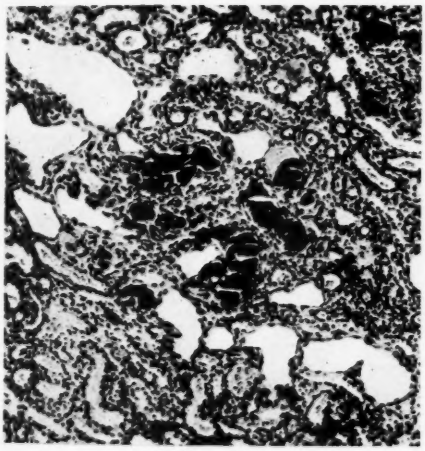

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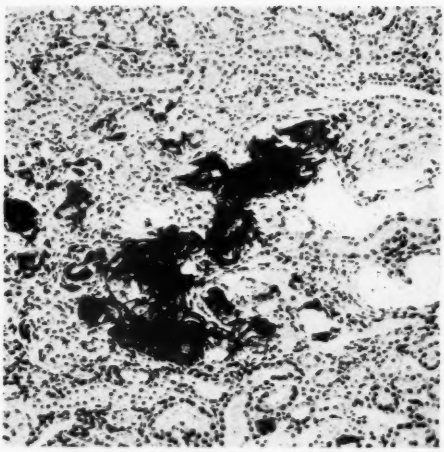

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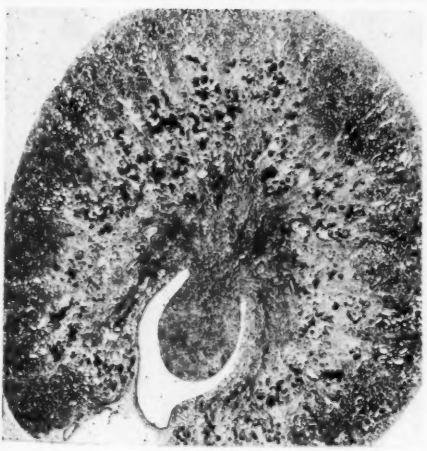

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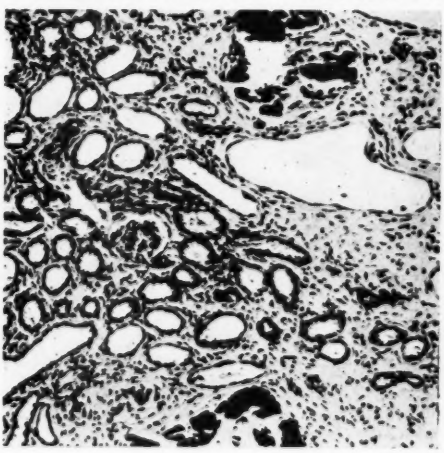

12

Morehead, Fishman, and Artom

Renal Injury Following Administration of Serine

EXTRACTS FROM
MINUTES OF THE MEETING OF THE COUNCIL

THE AMERICAN ASSOCIATION OF
PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND

MAY FIFTH, 1945

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Extracts from Minutes of the Meeting of the Council Held at Cleveland, Ohio, May 5, 1945

Present. President CANNON, DOCTORS FORBUS, GOODPASTURE, HAYTHORN, KARSNER, MORITZ, SOULE, WARREN, and WELLER.

The following were elected to membership in the Association:

LEWIS B. BATES
BERNARD BLACK-SCHAFFER
JASPER DIXON BUSH, JR.
REUBEN CARES
ISADORE NATHAN DUBIN
KENNETH MILO ENDICOTT
HORACE KERR GIFFEN
IRVING ISRAEL GOODOF
JUNE U. GUNTER
DESMOND E. O'C. MAGNER
WILLIAM AVISON MEISSNER
HENRY D. MOON
ROBERT PAGE MOREHEAD
EMMA S. MOSS
KARL T. NEUBUERGER
AUGUSTIN R. PEALE
GEORGE G. PROSKAUER
ENID KATHLEEN RUTLEDGE
HANS G. SCHLUMBERGER
HENRY SIEGEL
RUTH SILBERBERG
WRAY JOSEPH TOMLINSON
MAX WACHSTEIN
LOUIS JENRETTE ZELDIS

At their request, Drs. Arthur L. Amolsch, Averill A. Liebow, J. H. Fisher, and Bjarne Pearson were reinstated to membership.

It was voted to accept with regret the resignations of Drs. E. H. Hatton, J. W. Jobling, M. Lederer, H. F. Traut, H. R. Churchill, and E. C. Rosenow.

The deaths of the following members were recorded with deep regret: John Shaw Dunn, Louis A. Julianelle, Frank P. McNamara, Seaton Sailer, and Grover C. Weil.

Dr. Malcolm H. Soule was reelected Associate Editor of *The American Journal of Pathology* for a term of one year.

Dr. H. M. Zimmerman, whose term expires as a member of the Editorial Board of *The American Journal of Pathology,* was reelected for a term of six years.

Dr. Cannon renewed the invitation of the University of Chicago to meet there in 1946. It was voted that, government regulations permitting, the annual scientific sessions of the Association will be held at

the University of Chicago in the spring of 1946. It was voted to adhere to the original intention of the Council and to have as the topic of the symposium, "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis." Dr. Wiley D. Forbus, Professor of Pathology of Duke University, will be the referee.

It was voted to nominate Dr. Howard T. Karsner as representative of the Association in the Division of Medical Sciences of the National Research Council to succeed Col. Esmond R. Long at the expiration of his term in June, 1945.

HOWARD T. KARSNER, *Secretary*